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The Distribution of Substances similar to Gibberellic Acid in Higher Plants

BY

MARGARET RADLEY

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ABSTRACT

Gibberellic-acid-like substances have been found in extracts from all parts of seedlings of tall and dwarf peas and in mature seeds of wheat, French bean and tall and dwarf peas. They were present in amounts equivalent to 0·1–0·3 μg. gibberellic acid in 100 plants (F.W. 100–200 g.). Immature runner bean seed yielded larger quantities, equivalent to 0·25 μg. gibberellic acid per gram fresh weight, distributed between testas, cotyledons, and embryos.

INTRODUCTION

GIBBERELLIC acid (GA) has marked growth-promoting effects when it is applied to certain plants. In particular the growth of dwarf varieties of some species, such as pea (*Pisum*) (Brian and Hemming, 1955), French bean (*Phaseolus*) (Brian, Elson, Hemming, and Radley, 1954) or maize (Phinney, 1956), is greatly accelerated, while the tall varieties are affected slightly if at all. This suggests that there may be a substance with similar physiological activity occurring naturally in higher plants. If this were produced in greater quantity in tall plants than in dwarf forms it could explain the difference in response.

Extracts from higher plants, with biological properties which we can now recognize as being similar, were first described by Mitchell, Skaggs, and Anderson (1951). They extracted the immature seeds of bean (*Phaseolus*) with ether, and after evaporation of the solvent, applied the extract mixed with lanolin to seedling plants of the same variety. This stimulated considerable elongation of the actively extending internodes of the test plants.

More recently West and Phinney (1956) and Phinney, West, Ritzel, and Neely (1957) have found substances in the seeds of nine genera of flowering plants with physiological properties indistinguishable from those of the gibberellins. From paper chromatographic evidence they concluded that the 'gibberellin-like' substances they obtained were not chemically identical with any of the known gibberellins. Acetone extracts of the young inflorescences of *Brassica napus* were shown to have 'GA-like' activity, that is they had similar physiological effects to those produced by GA when applied to intact plants of various dwarf types (Lona, 1957). A preliminary report of the occurrence of such a hormone in pea seedlings was given by Radley (1956). In this paper an account is given of the extraction of such substances from various plant tissues.

[Annals of Botany, N.S. Vol. XXII, No. 87, July 1958.]

MATERIALS AND METHODS

General procedure. The procedure adopted involved extraction of the tissues with alcohol and subsequent purification. In preliminary experiments it was found that alcohol extracted considerably more active material than ether.

In order to follow the GA-like materials during paper chromatography of the partially purified extracts it was necessary to devise a new biological test for such activity. The response of wheat coleoptile sections to GA is very small (Brian, Hemming, and Radley, 1955), but it was shown by Hayashi and Murakami (1954) that sections of leaves of oat and barley seedlings would respond to very small amounts of gibberellin. Sections of the first leaf of etiolated wheat seedlings have been used in these experiments. As will be shown, the leaf section test is not completely specific to GA. Also, impurities in the extracts produced variations in both the Rf and the spread of the active substances on chromatograms. This was confirmed when a mixture of pea-shoot extract and GA was chromatogrammed. In some solvent systems the Rf of GA in the mixture was markedly different from its Rf when run on its own. To distinguish with certainty GA-like activity from that of the indole auxins the more cumbersome method was used of application to intact seedlings of genetically dwarf peas. Therefore a guide strip from each chromatogram was examined by the leaf section test, and fractions suspected to contain GA-like hormones were further tested by the pea plant assay. The methods of assay and extraction are described in more detail below.

Wheat-leaf section test. Wheat seeds (var. 'Victor') were soaked in distilled water overnight. The seeds were sown, embryo upwards, on surgical gauze covering the ends of 'perspex' cylinders. These were placed in 400 ml. beakers, which were filled with distilled water up to the level of the gauze. The beakers were covered with Petri-dish lids to maintain high humidity, and they were kept in the dark for 4 days at a constant temperature of 23° C.

The shoots were cut close to the seed. They were placed in a stainless-steel cutter and parallel razor blades cut 4 mm. sections through both coleoptile and leaf 4 mm. from the base of the shoot. The experimental reasons for choosing this particular section are presented later in this paper. The leaf sections, still enclosed in the coleoptile sections, will not grow satisfactorily if they are floated on the test solution unless the solutions are aerated by passing air through them. The most convenient way to grow them is to support them so that they are kept moist but are not actually immersed in the solution.

Chromatograms were tested by cutting them into a number of pieces, placing each piece in a depression in a 'perspex' block, and moistening with water. 0·25 ml. water was added to 5 cm.^{2} paper. Ten leaf sections were placed on each piece, and they were incubated in a moist atmosphere in the dark for 48 hours at 23° C. At the end of this time growth had almost ceased. The final length of the leaf sections was measured with the aid of a Hilger Inspection Enlarger (× 10 magnification).

In order to test solutions with wheat-leaf sections a method was used similar to that described by Audus and Thresh (1953) for sections of roots. A strip of filter paper was wrapped round a piece of microscope slide and placed in a 6 cm. diameter Petri-dish. Two ml. of the test solution was placed in the dish. Ten wheat-leaf sections were placed on the filter-paper wick and were incubated as described above.

Pea-plant assay. Dwarf peas (var. 'Meteor') were grown in pairs in 400 ml. beakers containing John Innes potting compost. Two weeks after sowing, when there were two pairs of leaves expanded, they were selected for uniformity. An initial measurement of shoot height was made from the base of the first true leaf.

In the earlier experiments the plant extracts were dissolved in a minimum of ethyl alcohol and were applied with a 0·1 ml. pipette to the lower pair of leaves. The alcohol was evaporated rapidly in a current of cold air. Later it was found that less damage was done to the leaves if the extracts were taken up in water. In this case the solution was allowed to dry naturally on the leaves.

The length of the shoots was measured twice a week, and during each 3- or 4-day period the increase in growth of the treated plants was compared with that of control plants.

Extraction. Five types of material were extracted in the experiments described below. Dry ungerminated seeds were ground in a mill before extraction, while germinated seeds and immature seeds merely had the testas removed and extracted separately from the seed. In all cases they were covered with 70 per cent. ethyl alcohol and allowed to stand overnight at room temperature. The solution was then filtered and the solid material was re-extracted for a further 3–4 hours. The second extract was filtered and the two solutions were bulked.

Pea seedlings were also extracted. They were grown in beakers of John Innes compost in a greenhouse. After harvesting they were stored in screw-top jars at $-15°$ C. They were extracted by first breaking down the frozen tissues into small pieces, and covering this material with absolute ethyl alcohol. The subsequent treatment was the same as for the seeds, except that the solid material was pressed dry after each extraction. The immature pods of French beans were extracted in the same way as the pea seedlings.

The ethyl alcohol was distilled off *in vacuo* at 36° C. The aqueous residue was filtered, adjusted to about pH 2·5, and extracted three times with equal volumes of ethyl acetate. The ethyl acetate solution was then extracted three times with quarter volumes of pH 6·2 buffer (KH_2PO_4 136 g.; KOH 24 g.; water 1 l.; see Borrow *et al.*, 1955). This was acidified to pH 2·5 and extracted three times with equal volumes of ethyl acetate. The solvent was dried by freezing out the water and filtering through a chilled funnel; it was then distilled off *in vacuo* at 36° C.

The residues were dissolved in alcohol and spotted on sheets of Whatman No. 1 chromatography paper. A small part of each sample was spotted on a 2 cm. wide strip, to act as a guide to the area of activity. The following solvent system was found to be the best for separating the zone of growth stimulation from the zones of inhibition: chloroform 20, ethanol 4, water 2, formic acid 1 part by volume. The guide strips were tested by the use of the wheat-leaf section test described above.

The sheet chromatograms were eluted with ethyl alcohol. The zones carrying active substances, as indicated by the wheat-leaf test on the guide strips, were eluted in the earlier experiments by allowing ethyl alcohol to run down the paper and drip off the end. A different method was used in later experiments. The paper was cut into pieces and placed in a cup-shaped container. Tubing connected to the base of this was bent to form a siphon. Ethyl alcohol was dripped into the container, siphoning off at intervals into a receiver. This proved to be a very efficient and trouble-free method. In both cases the alcohol was evaporated under reduced pressure. The eluted residues were tested for GA-like activity by application to intact dwarf pea plants.

RESULTS

Extension of wheat-leaf sections. As explained above, the wheat-leaf section test was used for preliminary detection of zones of GA-like activity on chromatograms. The response of different parts of the leaf to GA and to indolylacetic acid (IAA) is described below.

Wheat leaves about 5 cm. long grown as described have a zone of active extension approximately 1·5 cm. long. The basal 4 mm. is stimulated by GA and is slightly inhibited by IAA, but in practice it cannot be used for an assay as it is mechanically very weak and easily damaged. In addition the variability of response is too large to give reliable results.

The section 4–8 mm. from the base is the zone of greatest extension. This is the section used for testing chromatograms. Even in distilled water it extends by 5–6 mm. GA will give an increase of 7–8 mm., but unfortunately the stimulation is not proportional to the dose applied. The results given in Table I show that a significant increase in growth is given by 0·01 μg./ml. GA, but that increases in concentration up to 100 μg./ml. do not give any further significant increase. IAA stimulates growth only at much higher concentrations, and in some cases there may even be a depression of growth. In Table 1 1·0 μg./ml. IAA does not produce a significant increase in growth, whereas 10·0 μg./ml. does. There is a significant interaction between the two substances, mixtures producing a greater than additive increase in growth at some concentrations.

The third 4 mm. section (8–12 mm. from the base) reacts somewhat like the second section. The growth is less under all conditions, but it has a greater response to combinations of GA and IAA (Table 2). The fourth section extends very little (approximately 2 mm. in distilled water). It does not respond significantly to either GA or IAA alone, but there is a considerable increase in combinations of the two.

Similar results have been found by Hayashi and Murakami (1954) with sections from etiolated barley leaves and green oat leaves. Also it has been shown by Brian and Hemming (1957 *a*, *b*) that light-grown pea internode sections only respond to GA when IAA is also present. On the other hand sections from the stems of etiolated peas do respond to GA alone (Brian, Hemming, and Radley, 1955). The variation in response of the wheat-leaf tissue with the stage of extension it has reached may perhaps be correlated with the level of endogenous IAA in the cells.

TABLE 1

The Final Length in mm. of Sections (initially 4 mm.) of the First Leaf of Dark-grown Wheat Seedlings cut 4 mm. from the Base, after Incubation for 48 hours in Various Concentrations of Gibberellic Acid and Indolylacetic Acid

	GA	0	0·01	0·1	1·0	10·0	100 μg./ml.
IAA	0	9·40	10·07	10·11	10·21	10·33	10·46
	1·0	9·73	9·88	10·29	10·55	10·72	11·17
	10 μg./ml.	10·05	10·04	10·52	11·14	10·27	10·23

Sig. diff. (P = 0·05) 0·42, (P = 0·01) 0·56

Analysis of variance

Effects	Sum of squares	Degrees of freedom	Variance
GA treatments .	78,708	5	15,742*
IAA treatments	12,988	2	6,494*
Interaction .	41,368	10	4,137*
Error . .	47,445	54	879

* Variance ratios significant at P = 0·01

Since it has been reported (Miller, 1956) that kinetin induces expansion of bean-leaf tissue, but inhibits extension of sections of etiolated pea stems, the 4–8 mm. wheat-leaf sections have been grown in solutions of kinetin both with and without GA or IAA (Table 3). Kinetin alone had no effect on growth at low concentrations, but at 100 μg./ml. it depressed growth slightly. The stimulation produced by 1·0 μg./ml. GA was reduced by 10 or 100 μg./ml. kinetin. IAA alone somewhat reduced extension in this experiment, but in the presence of 10 or 100 μg./ml. kinetin there was a marked depression of growth. Thus kinetin and GA can be readily distinguished by this test.

Extracts of plant tissues. Extracts which induced accelerated growth of dwarf pea seedlings were obtained from all the plant tissues examined. Several days after application of such extracts the test plants developed the long internodes and pale green foliage characteristically produced by gibberellic acid and the gibberellins. The results are discussed in greater detail below.

Pea seedlings. The results of two experiments with tall (Improved Pilot) peas are presented in Table 4. An increase in growth rate to approximately 130 per cent. of that of control plants is significant. In the first of these the shoots of seedlings were extracted 17, 22, and 30 days after sowing, the shoots being divided into an upper portion consisting of that part of the stem still capable of further extension, with attached leaves, and a lower portion comprising the remainder of the shoot, viz. the mature part of the stem with

Table 2

The Final Length in mm. of Sections (initially 4 mm.) of the First Leaf of Dark-grown Wheat Seedlings cut 8 mm. or 12 mm. from the Base, after Incubation for 48 hours in Various Concentrations of Gibberellic Acid and Indolylacetic Acid

8–12 mm. SECTIONS

GA	0	0·1	1·0	10·0 μg./ml.
IAA 0	7·54	8·06	8·59	8·17
1·0	7·57	8·42	8·96	9·13
10·0 μg./ml.	7·72	9·24	9·61	9·75

Sig. diff. ($P = 0·05$) 0·38, ($P = 0·01$) 0·52

Analysis of variance

Effect	Sum of squares	Degrees of freedom	Variance
GA treatments	121,439	3	40,480*
IAA treatments	59,230	2	29,615*
Interaction	17,463	6	2,911*
Error	12,477	24	520

* Variance ratios significant at $P = 0·01$

12–16 mm. SECTIONS

GA	0	0·1	1·0	10·0 μg./ml.
IAA 0	6·04	5·92	6·35	6·25
1·0	5·78	5·99	6·79	7·09
10·0 μg./ml.	6·21	6·95	7·46	7·21

Sig. diff. ($P = 0·05$) 0·34, ($P = 0·01$) 0·45

Analysis of variance

Effects	Sum of squares	Degree of freedom	Variance
GA treatments	49,088	3	16,363*
IAA treatments	41,475	2	20,738*
Interaction	16,275	6	2,713*
Error	9,513	24	396

* Variance ratios significant at $P = 0·01$

Table 3

The Final Length in mm. of Sections (initially 4 mm.) of the First Leaf of Dark-grown Wheat Seedlings cut 4 mm. from the Base, after Incubation for 48 hours in Various Concentrations of Kinetin with and without the Addition of Gibberellic Acid or Indolylacetic Acid

Kinetin	0	1·0	10·0	100·0 μg./ml.
Water	9·91	9·96	9·86	9·58
1 μg./ml. IAA	9·39	9·42	8·98	8·60
1 μg./ml. GA	11·45	11·43	11·13	11·10

Sig. diff. ($P = 0·05$) 0·29, ($P = 0·01$) 0·39 attached leaves. All these extracts show obvious GA-like activity; there was some indication that the amount of active material increased with increasing age and size of the plant. In the second experiment plants of one age only were extracted, the shoots being divided into upper and lower portions as before but in addition stems and leaves were separately extracted. Material with GA-like activity was found in each of these parts.

TABLE 4

Growth-rate of Meteor Pea Seedlings, expressed as Percentage of that of Untreated Seedlings, after Application of Extracts from Improved Pilot Seedlings

[In Expt. 1 the extract from 45 plants was applied to each test plant, in Expt. 2 that from 125 plants]

		Period of observation (days after treatment)		
Age of plant	Tissue	0–4	4–8	8–11
Experiment 1				
17 days	Whole shoot	148	100	100
22 ,,	Upper shoot	152	128	100
,, ,,	Lower shoot	187	150	100
30 ,,	Upper shoot	230	172	106
,, ,,	Lower shoot	239	311	153
		Period of observation (days after treatment)		
		0–2	2–4	4–7
Experiment 2				
28 days	Upper stem	110	217	154
,,	Upper leaves	160	225	154
,,	Lower stem	130	192	133
,,	Lower leaves	110	175	125

A growth response of test plants of the same order as those presented in Table 4 can be obtained by applying 0·1 μg. GA to each plant. Thus in Expt. 2 (Table 4) the total active material in 125 plants (total fresh weight about 250 g.) is approximately equivalent in activity to 0·4 μg. GA. This may well be an underestimate of the quantity of active material actually in the extracts, as toxic substances in the samples certainly affected the growth of some test plants. Despite this, it seems likely that the amount of active material in Improved Pilot peas is, in terms of GA equivalent, extremely small.

A comparison of dwarf (Meteor) and tall (Improved Pilot) peas is summarized in Table 5. There is little evidence of a difference between the two varieties in content of GA-like materials.

Extracts with GA-like activity were also obtained from roots of Meteor and Improved Pilot peas and from shoots of etiolated plants of both varieties grown in complete darkness for 9 days. Extracts of the shrunken cotyledons still attached to the etiolated shoots were also active.

Seeds. Active extracts were also obtained from mature dry seeds of Wheat (var. Victor), French bean (var. Canadian Wonder), and dwarf (Meteor) and tall (Improved Pilot) peas. Extracts from 25 g. seed contained materials with activity equivalent to about 0·1 μg. GA. Active substances were also found in bean and pea seeds 1–5 days after germination; such substances were present in both cotyledon and testa. The amounts in testa and cotyledons appeared to be similar, if samples from the same number of seeds were compared.

TABLE 5

Growth-rate of Meteor Pea Seedlings, expressed as Percentage of that of Untreated Seedlings, after Application of Extracts from Meteor (dwarf) and Improved Pilot (tall) Pea Seedlings

[The extract from 125 plants was applied to each test plant]

Tissue	F.W. of parts of 500 plants mg.	Variety	Period of observation (days after treatment) 0–2	2–4	4–7	7–11
Upper stem	110	Meteor	125	146	121	134
,,	149	I. Pilot	200	191	138	121
Lower stem	195	Meteor	137	155	134	123
,,	345	I. Pilot	118	136	103	102
Upper leaves	86	Meteor	Extract contaminated			
,,	80	I. Pilot	118	164	162	100
Lower leaves	263	Meteor	175	154	121	102
,,	234	I. Pilot	175	173	148	123

Immature runner bean seeds (of 2–6 mm. length) contained notably higher concentrations of GA-like material, estimated to be equivalent to at least 0·25 μg. GA per gram fresh weight of seed. The extracts prepared from this material had a greater and more prolonged effect on the growth of the test plants (Table 6). The active material was present in cotyledons, testas, and

TABLE 6

Growth-rate of Meteor Pea Seedlings, expressed as Percentage of that of Untreated Seedlings, after Application of Extracts of Immature Runner Bean Seed

[Total fresh weight of seed 350 g. After extraction weight of residues: embryos 1·015 g., cotyledons 85·3 g., testas 100·8 g.]

Tissue	Percentage of total extract applied to each test plant	Period of observation (days after treatment) 0–3	3–6	6–10	10–13	13–17
Embryos	10	282	373	438	390	263
	2	291	347	283	266	200
	0·25	200	143	150	133	107
	0·025	100	100	100	100	100
Cotyledons	10	300	313	342	500	410
	2	255	380	467	529	424
	0·25	270	436	567	573	277
	0·025	250	243	217	161	120
Testas	2·5	282	353	420	524	460
	1·0	291	373	475	595	416
	0·4	310	436	550	529	333
	0·04	160	243	269	211	158

embryos. The amounts extracted per gram of tissue from these three fractions were roughly equal, if estimated on the residual weights measured after extraction. That means a single embryo contained only a small part of the active material in the whole seed. Extracts of the immature pods from which the immature seeds were obtained also showed GA-like activity, of the same order as shoot tissue.

DISCUSSION

The experiments described above show that substances with biological activity similar to that of gibberellic acid occur in all parts of pea plants including the fruit and seed. There is not enough evidence from these experiments to show the quantitative distribution between the different parts of the plant, but the results suggest that, apart from the immature seed, there are not marked differences in concentration. This is in contrast with the results found for IAA, where there appears to be a high concentration of free auxin in the stem apex, and progressively decreasing concentrations in those parts of the plant farther from the apex. IAA is also found in plants in two forms, a 'free' diffusible auxin, and a 'bound' form, which requires prolonged extraction. There is not yet any evidence whether or not the GA-like substances occur in two forms.

The results of the experiments given above suggest a concentration of the order of 1–3 microgram equivalents of GA in 1 kilogram of shoot tissue. This is somewhat lower than results quoted for IAA. For example, van Overbeek *et al.* (1947) found a concentration of 11 μg. IAA equivalents per kilogram of young pineapple leaves, while Henderson and Bonner (1952) found 74 μg. IAA equivalents per kilogram of sunflower stems.

There is a much higher concentration of GA-like material in immature bean seeds, but the amount falls to a level comparable with that of the growing plant in the mature seed. Mitchell, Skaggs, and Anderson (1951) found that the amount of ether-extractable hormones with this type of activity increased in bean seeds up to about 8 days after pollination and then decreased rapidly to an immeasurable amount on about the fifteenth day. A similar result was reported by Avery, Berger, and Shalucha (1942) for IAA-type auxins in maize kernels. At pollination the total auxin (including a precursor) present in maize kernels was very small. There was a rapid increase for 1 to 3 weeks from pollination followed by a decrease to the dormant stage. The maximum concentration was estimated to be 0·3–0·5 mg. IAA per gram dry weight, although the majority of this was in the form of a precursor. This result can be compared with the figure of 0·25 μg. per gram fresh weight given above for GA-like substances in immature bean seed. This is a low estimate and the true value may be higher, but even so it is of quite a different order from the IAA concentration.

Brian (1957) suggested that the difference in growth-rate between tall and dwarf peas may be due to the presence in the former of a growth-promoting substance similar to GA. The possibility that there may be a higher level of

GA-like substances in tall varieties than in dwarfs is not excluded by the results given here, which are only semi-quantitative, although one would expect a large difference to be obvious. It may be that in dwarf plants the substances are present in a form unable to exert any influence on cell growth, or that there may be inhibitors present which prevent it acting.

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SUMMARY

1. In order to find GA-like materials on chromatograms a new biological test has been used in which sections of the first leaf of etiolated wheat shoots were incubated on pieces of the chromatograms. They responded to GA by increased growth. Kinetin does not stimulate growth, and IAA only does so at relatively high concentrations.

2. Extracts of plant tissues have been examined by means of this test and by application to intact dwarf pea plants. Substances have been found with similar biological properties to those of GA.

3. GA-like substances have been found in quantities of the order of 0·1–0·3 μg. in 100 plants (F.W. 100–200 g.) in all parts of tall and dwarf pea seedlings and in mature seeds of tall and dwarf peas, wheat, and French beans. There is no evidence of a difference between tall and dwarf varieties of peas in content of GA-like materials.

4. Larger quantities of these active substances were found in immature runner bean seeds. A minimum of 0·25 μg. GA equivalent per gram of seed was found to be distributed between testas, cotyledons, and embryos.

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Studies in the Physiology of Parasitism

XXV. Some Properties of the Pectic Enzymes secreted by *Pythium debaryanum*

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ABSTRACT

The pectic enzymes of *Pythium debaryanum* have been compared with those from two other soft-rot causing organisms, *Erwinia aroideae* and *Botrytis cinerea*, by their effects (viscosity reduction, acid production, and reducing power) on 1 per cent. solutions of (*a*) high methoxyl pectin, (*b*) sodium polypectate, and (*c*) sodium pectate (2 types). The *Pythium debaryanum* preparation differed particularly in giving no increase in reducing power or evidence of galacturonic-acid-like derivatives. It macerated the walls of potato-tissue freely but had no polygalacturonase or pectinesterase activity.

INTRODUCTION

IN another paper of this series (Gupta, 1956) the effects of cultural conditions on the secretion of pectic enzymes by the plant pathogen *Pythium debaryanum* Hesse were described in some detail. It was found that active culture filtrates were obtained only when the carbon and nitrogen constituents of synthetic media were carefully balanced. Enzyme secretion was increased when sodium chloride was added and when the medium, containing glucose as the carbon source, was autoclaved at relatively high pH values. Less active filtrates were obtained when glucose was replaced by a high methoxyl pectin. *P. debaryanum* behaved differently in these respects from a number of other plant pathogens which secrete pectic enzymes abundantly in a variety of synthetic media, and which produce as active, or much more active, filtrates with pectic materials as the carbon source. In view of these differences some of the properties of the pectic enzymes produced by *P. debaryanum* have been studied and compared with those of the enzymes secreted by *Erwinia aroideae* and *Botrytis cinerea*, two organisms which also cause soft-rots of plant tissue.

MATERIALS AND METHODS

The fungi used were *P. debaryanum* Hesse isolated from a soft-rotted potato tuber, *B. cinerea* Pers. from a lettuce seedling, and *E. aroideae* (Townsend) Holland from a tobacco plant.

Enzyme solutions were prepared from tubers rotted by *P. debaryanum*, and from cultures of the three organisms on synthetic media. King Edward potato tubers were well washed and surface-sterilized by immersing them in

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a solution containing about 1 per cent. available chlorine for 30 minutes, and then allowing them to dry. Cylinders, 5 mm. diameter and 5 mm. long, were removed from the tubers and in the cavities were placed discs taken from the edge of growing colonies of *P. debaryanum* on potato extract agar. The top of the cavity was covered with a square of sterile filter paper which was sealed and stuck to the tuber with 'Vaseline'. The tubers were incubated at 25° C; a rapidly spreading rot developed from the majority of the inoculations, and solutions containing pectic enzymes were prepared from the juice which was readily extracted from the rotted tissue.

P. debaryanum was grown in a medium containing glucose 3·0%, peptone 0·45%, asparagine 0·45%, K_3PO_4 0·33%, $MgSO_4.7H_2O$ 0·2% and NaCl 0·1%. Ten discs, 0·5 cm. diameter, from the edge of a growing colony on water agar, were placed in 20 ml. of the medium contained as a shallow layer in 12 oz. bottles.

B. cinerea. The medium contained glucose 2·0%, asparagine 0·5%, KH_2PO_4 0·1%, $MgSO_4.7H_2O$ 0·05%; 30 ml. in 12 oz. bottles were seeded with the spores from the surface of a young, vigorously sporing culture on potato extract agar in a 2 × 15 cm. tube.

E. aroideae. Excess $CaCO_3$ was added to the medium described for *B. cinerea*; 30 ml. in 12 oz. bottles were seeded with 0·025 ml. of a 48-hour broth culture on the same medium.

Cultures of the three organisms were grown at 25° for 5 days; cell-free filtrates, prepared from these cultures by centrifuging at 10,000 r.p.m. for 10 minutes, were stored under toluene at 4° for short periods or at −18° for longer periods.

Pectic materials were kindly supplied by Exchange Lemon Products Company, California, and were derived from citrus products. These materials were purified by washing in 60 per cent. ethanol containing 0·1 N HCl, in 60 per cent. ethanol until free of chloride, and finally in ethanol; they were dried at room temperature and then under reduced pressure over calcium chloride at 50°.

The materials were high methoxyl pectin containing 10·4 per cent. methoxyl (71 per cent. esterified); sodium polypectate (sodium ammonium pectate) stated to have an estimated molecular weight of 2–300,000; pectic acid which was essentially free of methoxyl groups. Solutions of sodium pectate were made by slowly adding 0·1 N NaOH to a suspension of pectic acid to give a final reaction near neutrality. Sodium pectate solutions were also prepared from solutions of pectin by methods described later.

The methods used for measuring the activity of different pectic enzymes have been described in earlier papers of this series (Wood, 1955 ; Singh and Wood, 1955). Briefly, they were as follows:

Protopectinase. Activity was assessed in terms of the time (Reaction Time) taken for discs of turgid potato-tuber tissue, 0·5 mm. thick and 8 mm. diameter, to lose coherence under slight tension after immersion in the test solution.

Pectinesterase. Activity was measured as the rate of liberation of carboxyl groups from pectin solutions.

Polygalacturonase. The increase in the number of reducing groups was measured.

Viscosity-reducing enzyme. The rate of loss of viscosity of solutions of pectic substances under standard conditions was measured.

All enzyme activities were measured at 25°. Further details of these and other methods will be given later.

RESULTS

Action of culture filtrates on different pectic substances

One per cent. solutions of the following substances were used. (*a*) High methoxyl pectin. (*b*) Sodium polypectate. (*c*) Sodium pectate prepared by neutralizing pectic acid. (*d*) Sodium pectate prepared by de-esterifying (*a*) with a pectinesterase preparation from fresh tobacco leaves; liberated carboxyl groups were neutralized with 0·1 N NaOH for 8 hours after adding a small quantity of a concentrated pectinesterase preparation; the mixture was kept overnight under toluene at 4°; more enzyme was then added and alkali added until de-esterification was complete. Although the pH of the mixture was not allowed to rise above 7·5 during de-esterification, there was some degradation because the relative viscosity of the solution finally obtained was only some 60 per cent. that of the original pectin solution.

The enzyme/substrate mixtures were: 5 ml. of (*a*), (*b*), (*c*), (*d*), or (*e*); sufficient NaOH or HCl to bring these solutions to pH 8·3 for *E. aroideae* and *P. debaryanum* and pH 6·0 for *B. cinerea*; 1·0 ml. KH_2PO_4/Na_2HPO_4 buffer (M/10) at pH 8·3 or 6·0; water to 8·0 ml.; 2·0 ml. cell-free filtrates from cultures of the three organisms.

The viscosities of the mixtures were measured at intervals after adding the enzyme solutions. The results are given in Table 1 in which the loss in viscosity after different times is expressed as a percentage of the total possible loss.

The filtrates from cultures of *E. aroideae* and *B. cinerea* rapidly degraded solutions of each of the four substrates. Solutions of pectin and sodium polypectate were degraded at about the same rate by the two preparations except in the earliest stages when the bacterial preparation was slightly the more active. But with the pectates, the *E. aroideae* preparation was more active on the one derived from pectic acid, whereas the *B. cinerea* preparation was more active on the one obtained by enzyme de-esterification of pectin.

An unexpected result was obtained with the *P. debaryanum* preparation which degraded the pectin solution almost as rapidly as did the other two preparations. But it did not reduce the viscosity of the two pectate solutions and only very slightly reduced the viscosity of the sodium polypectate solution. This result was the more striking in view of the fact that one of the solutions of sodium pectate had been derived directly from a sample of the pectin solution which was so rapidly degraded.

TABLE 1

Activity of Viscosity-reducing Enzymes of E. aroideae, B. cinerea, *and* P. debaryanum

		% viscosity loss after (min.)				
Substrate	Enzyme solution	1	2	4	10	20
Pectin	*E. aroideae*	61	70	80	87	93
	B. cinerea	41	54	72	90	96
	P. debaryanum	32	40	51	68	78
Sodium polypectate . .	*E. aroideae*	42	51	69	87	92
	B. cinerea	22	45	65	86	95
	P. debaryanum	0	0	1	2	2
Sodium pectate (alkali) .	*E. aroideae*	*	23	53	87	100
	B. cinerea	*	12	23	65	70
	P. debaryanum	0	0	0	0	0
Sodium pectate (enzyme) .	*E. aroideae*	*	8	19	52	80
	B. cinerea	26	44	62	83	92
	P. debaryanum	0	0	0	0	0

* Temporary increase in viscosity caused, presumably, by partial precipitation with cations in culture filtrates.

After incubation of the enzyme-substrate mixtures under toluene for 24 hours at 25° the reducing values of 5 ml. samples were measured by the Willstätter–Schudel hypodiodite method. The results are shown in Table 2 and are expressed as the per cent. hydrolysis of polyuronide present in the sample.

TABLE 2

Polygalacturonase Activity of Filtrates of Cultures of E. aroideae, B. cinerea, *and* P. debaryanum

	% hydrolysis		
Substrate	*E. aroideae*	*B. cinerea*	*P. debaryanum*
Pectin	28	68	3
Sodium polypectate	41	77	5
Sodium pectate (alkali) . . .	45	64	3
Sodium pectate (enzyme) . . .	38	48	0

The *E. aroideae* and *B. cinerea* preparations had about the same activity with sodium pectate prepared by enzyme de-esterification, but with the other three substrates, and particularly with pectin, the fungal preparation was the more active. Each substrate was, however, substantially degraded. In contrast, the action of the *P. debaryanum* preparation caused practically no increase in the reducing power of the mixtures. This was expected for sodium polypectate and the two pectates but was not expected for pectin in view of the rapidity with which the viscosity of a pectin solution was reduced.

Samples from the mixtures which had been incubated for 24 hours were also examined by descending paper chromatography with a butanol-acetic acid-water (4:1:5 by volume) mixture. The chromatogram was developed for 4 days at room temperature to get good separation of the components and sprayed with a benzidine-trichloroacetic acid mixture before heating at 90–100°. Glucose and galacturonic acid (50 μg. each) were used as markers and appeared as prominent spots on the developed chromatogram. A prominent galacturonic acid spot was obtained with each substrate which had been treated with the *B. cinerea* preparation but there was little evidence of intermediate compounds. The *E. aroideae* preparation did not produce galacturonic acid from any of the substrates; two spots intermediate between the galacturonic acid position and the base-line were produced from pectin and sodium polypectate, and one intermediate spot, at the same level, from each of the pectates. In contrast, the *P. debaryanum* preparation did not produce, from any of the substrates, substances which moved from the point of application. This confirms the results given in Table 2.

Some chromatograms were sprayed before the solvent front had reached the bottom of the paper; no spots appeared below the level of the glucose.

The culture filtrates used in the above experiments macerated discs of potato-tuber tissue in the following times: *E. aroideae* at pH 8·3, 12–15 minutes; *B. cinerea* at pH 6·0, 20–25 minutes; *P. debaryanum* at pH 8·3, 30–40 minutes.

In a further experiment the activity of the *P. debaryanum* preparation was increased five times by using a solution of an acetone precipitate from a culture filtrate. This preparation reduced the viscosity of a 0·5% solution of pectin by 50% after 1 minute, by 70% after 2 minutes, 85% after 4 minutes, 95% after 10 minutes, 97% after 20 minutes, and 98% after 60 minutes. The percentage hydrolysis of 5 ml. samples taken 2·5, 5·5, and 24 hours after adding the enzyme was 4, 7, and 12. After 48 hours a solution of Pectinol 41, with a high polygalacturonase and low pectinesterase activity, which had been dialysed for 48 hours against 0·1 M NaCl with a slight increase in activity, was added to part of the mixture which was then incubated for a further 24 hours. The percentage hydrolysis of 5 ml. samples (after correction for dilution) was 67. In contrast the figure for that part of the mixture to which Pectinol had not been added was only 15. This showed that the products remaining in the mixture after 24 hours' incubation with the *P. debaryanum* preparation were rapidly degraded by Pectinol. These results were confirmed when samples of the mixtures were examined by chromatography in the way described above. Spraying with the benzidine mixture before the solvent front had reached the bottom of the sheet revealed no spots below the level of glucose. Samples from the substrates incubated with the *P. debaryanum* preparation for 24 and 48 hours gave a very faint spot at the level of galacturonic acid and an equally faint spot about midway between galacturonic acid and the base line. After Pectinol had been added the intermediate spot disappeared but a well-developed galacturonic acid spot appeared. It was also confirmed that the mixtures after treatment with the *P. debaryanum* preparation did not interfere with the running of *added* galacturonic acid.

These results showed clearly that the enzymes secreted by *P. debaryanum* were able to cause only a limited degradation of pectin in solution and that the products which were produced were of sufficiently high molecular weight to move very slowly on the paper when this was eluted with the butanol-acetic acid-water mixture.

Action on pectinic acids of different methoxyl content. A series of solutions of pectinic acids of different methoxyl content were prepared by slowly adding to a solution of a high methoxyl pectin appropriate quantities of NaOH. The viscosity changes in 0·5 per cent. solutions of these materials containing 20 per cent. of a *P. debaryanum* culture filtrate and 0·02 M phosphate buffer at pH 8·0 were followed. The results, expressed as loss of viscosity (as a percentage of the total possible loss), are given in Table 3.

TABLE 3

Effect of P. debaryanum *Culture Filtrates on Pectinic Acids of Different Methoxyl Contents*

	% viscosity loss after (min.)				
% methoxyl	1	2	4	10	20
10·4	31	50	67	80	88
8·1	21	37	52	69	77
5·8	6	13	27	40	45
3·6	0	1	1	8	11
1·4	0	0	2	6	9
0	0	0	0	0	0

The activity of the viscosity-reducing enzyme was reduced by about 50 per cent. when the methoxyl content of the pectinic acid was reduced to about half that in the original pectin. There was only a very slow reduction in viscosity when the methoxyl content was reduced to 3·6 per cent. and the enzyme preparation had no effect on the viscosity of the de-esterified pectin solution.

A filtrate from a culture of *E. aroideae* used in a similar experiment rapidly reduced the viscosity of each of the solutions.

A distinguishing feature of the hydrolytic pectic enzymes produced by *P. debaryanum* in liquid media is, therefore, that the substrate is highly methoxylated pectin; in this respect they differ from the pectic enzymes produced by most other fungi. It may be mentioned here that filtrates which reduced rapidly the viscosity of pectin solutions had very little pectinesterase activity. The addition of pectin to culture media did not increase the pectinesterase content of the culture filtrates. This enzyme therefore played no part in the activity of the viscosity-reducing enzymes in the tests described above.

Effect of concentration on activity of the viscosity-reducing enzyme. 360 ml. of a cell-free filtrate from cultures of the fungus on the synthetic medium described above were freeze-dried to give a fluffy, light-brown coloured product which after being dried over $CaCl_2$ weighed 3·12 g. When this was tested on a 0·5 per cent. solution of pectin containing 0·02 M phosphate buffer at pH 8·3 it was found that the dried product retained some two-thirds of the original activity. The powder was stored at −18° and retained its activity for many months. It was used to determine the effect of concentration on the activity of the viscosity-reducing enzymes. Different weights were added to solutions containing 0·5 per cent. pectin, sufficient NaOH to bring the pH to 8·3, and 0·02 M phosphate buffer at the same pH. The viscosity changes during the 20-minute period after adding the enzyme are given in Table 4.

TABLE 4

Effect of Enzyme Concentration on Activity of Viscosity-reducing Enzymes

Enzyme concentration	Loss of viscosity (as % of total possible) after (min.)				
	1	2	4	10	20
100	73	86	93	99	100
40	53	70	85	94	98
20	36	54	70	87	93
8	25	37	48	68	82
4	16	23	36	53	68

It will be seen that for the range of viscosity changes represented in the table, the time taken for an enzyme preparation to produce a certain reduction in viscosity was inversely proportional to the enzyme concentration. This relationship was used to estimate the effect of different conditions and treatments on enzyme activity which are summarized below.

Effect of pH on enzyme activity. The test mixtures contained 0·5 per cent. pectin, NaOH or HCl to bring to the required final pH, 0·02 M phosphate at the same pH, and 20 per cent. cell-free culture filtrate. Precise measurements of activity were not obtained because at pH values away from the optimum the viscosities of the mixtures were reduced very slowly. It was clear, however, that the pH optimum was near 9·0, at least for the early stages of hydrolysis. It was difficult to determine the effect of higher pH values on activity because of the degradation of the pectin which occurred spontaneously at these high values, but activity was reduced rapidly below pH 9·0, e.g. at pH 7·8 it was reduced by about 75 per cent. and at pH 6·5 the viscosity-reducing enzyme was almost inactive.

Effect of dialysis. 15 ml. of enzyme solutions were placed under toluene in cellophane bags and dialysed against tap water for 3 hours, and for two periods of 24 hours at 4° in two lots of 1 l. distilled water. Allowance was made for the dilution following dialysis when the activities of the dialysed solutions were measured. Three types of enzyme solutions were used: (*a*) cell-free filtrates from cultures on synthetic media, (*b*) juice (*x* ml.) from rotted tuber tissue, (*c*) residue from (*b*) extracted with *x* ml. 0·5 M Na_2HPO_4; (*b*) and (*c*) were made cell-free by centrifuging.

After dialysis of (*a*) a precipitate had formed which did not dissolve after 30 minutes at 25°. The cleared dialysate had about a third of the activity of the undialysed solution. The addition of a suspension of the precipitate to the substrate showed that it contained about a fifth of the original activity. The loss of activity of the dialysed solution was not regained when autoclaved undialysed solutions were added,

A much more striking loss of activity following dialysis was obtained with solutions (*b*) and (*c*). Inactivation was almost complete in the absence of salts and there was only a twentieth of the original activity when autoclaved dialysed solutions were added.

Thermal inactivation.—5 ml. of untreated filtrates were placed in thin-wall glass tubes and kept in water-baths at different temperatures for 5 minutes and then transferred to ice-water. The viscosity-reducing activity was measured shortly afterwards. Inactivation began at 45°, was almost complete at 50°, and complete at 55° and at higher temperatures.

Purification. Enzyme solutions could not be purified by dialysis because of the irreversible loss of activity which occurred. But enzyme solutions could be concentrated and partially purified by precipitation with acetone. Cell-free filtrates from cultures on synthetic media were cooled to about 4° and mixed quickly with acetone at about −18°. A flocculent precipitate formed rapidly and was easily collected by centrifuging. An extract of this precipitate with 0·2 M phosphate buffer at pH 8·0 contained about 50 per cent. of the original viscosity-reducing activity. Acetone at final concentrations of 50, 67, and 75 per cent. produced much the same results.

Attempts were also made to adsorb the viscosity-reducing enzyme on different substances. Before this was done the stability of solutions of the enzyme at low pH values was studied. It was found that there was little loss of activity when filtrates were kept at pH 4·5 for 2 hours.

A diatomaceous filter-aid (Hyflo Supercel) and a cellulose powder (Balston, Standard grade) adsorbed about two-thirds of the activity from filtrates maintained at pH 4·5. The supernatant fluid from filtrates at pH 4·5 treated with kaolin was inactive; the enzyme was not eluted from the kaolin by 0·5 M Na_2HPO_4, 0·3 and 1·0 saturated $(NH_4)_2SO_4$ at pH 7·0, 0·2 M CH_3COONH_4, 0·5 M KH_2PO_4, 0·2 M Na_2HCO_3, 0·2 M K_3PO_4; about a fifth of the original activity was recovered after elution with 0·1 M Na_2CO_3 and 0·2 M $Na_2B_4O_7$.

When the kaolin, with the adsorbed enzyme, was first washed in water, resuspended in water, and the suspension added to the pectin substrate, the viscosity of the mixture was reduced at about one-fifth of the rate produced by the untreated filtrates. This showed that the specific substrate could desorb, at least partially, the enzyme from the kaolin. No other eluants were tested but it is apparent that if a suitable one were found the viscosity-reducing enzyme could be substantially purified relatively easily.

Viscosity-reducing activity of tuber extracts. In the experiments described above, with one exception, filtrates from cultures on synthetic media were used. Solutions of high viscosity-reducing activity could also be obtained from tuber tissue rotted by the pathogen. When tubers were inoculated with the pathogen great care was taken to avoid contamination, particularly because the water-soaked rot caused by the fungus was readily invaded by bacteria which did not invade or spread through healthy tuber tissue. When contamination was suspected, the tuber was rejected.

The fungus spread rapidly from the inoculum; the medulla of the tuber was attacked more readily than the cortex. The skin always remained intact and in the later stages was the only part of the tuber not affected. The rotted tissue was soft and exuded liquid freely; when freshly exposed it was only faintly discoloured but it darkened rapidly on exposure to the air. In the earlier stages of attack, the rotted tissue was clearly delimited from healthy tissue.

Rotted tissue was removed from the tuber and placed immediately at −18°; the enzyme activity of this frozen tissue altered little over many months.

A hand press was used to extract juice from thawed rotted tissue through four layers of muslin; the juice was clarified by centrifuging and was finally dark brown in colour with a pH of 6·8–7·0. Water extracts of the residue had little activity but extracts with 0·5 M Na_2HPO_4 had about the same activity as the original extract. The original and phosphate extract behaved similarly and reduced the viscosity of pectin solutions at about the same rate as did culture filtrates. The extracts had little effect on the viscosity of solutions of sodium polypectate and sodium pectate, and were most active at about pH 9·0. The effect of dialysis has already been described.

Except for slight differences after dialysis, the viscosity-reducing enzyme produced in culture and in tubers behaved similarly.

Activity of other pectic enzymes. Pectinesterase could not be detected in filtrates from cultures on synthetic media or on a potato extract; both filtrates had high viscosity-reducing activity. But pectinesterase was readily detected in cell-free preparations from rotted tuber tissue. The activity of different preparations varied considerably but it was generally quite high. The results of a typical experiment are shown in Table 5. The enzyme-substrate mixture contained 1 per cent. pectin, 20 per cent. by volume of the extracts; the activity was measured in the pH range 6·0–8·0.

TABLE 5

Pectinesterase Activity of Rotted Tuber Extracts

Time (hrs.)	0·5	1	1·5	2	2·5	3	48
% de-esterification	19	28	42	51	60	73	100

Tuber extracts having pectinesterase activity reduced the viscosity of solutions of pectin but not of sodium polypectate or pectate, so it seemed unlikely that the pectinesterase had been produced in the tuber by a contaminant unless this contaminant did not produce viscosity-reducing enzymes or produced enzymes with properties very similar to those produced by *P. debaryanum*. Furthermore, juice pressed from tubers which had been frozen for 24 hours and then thawed, and 0·2 M Na_2HPO_4 extracts of the residue, under the same conditions of testing each had only a third of the activity of extracts from rotted tissue. It is possible that maceration of the tissue by the fungus brings it into a state in which the pectinesterase present is more easily and efficiently extracted. Alternatively, the fungus itself may produce this enzyme in tubers although it does not do so in culture on liquid media. Further work is necessary to decide this point.

Protopectinase. Filtrates from cultures on synthetic media or on potato extract, and extracts from rotted tuber tissue readily macerated discs of tuber tissue. Macerating activity was low at pH 5·0 and increased rapidly at higher pH values to a maximum at about pH 9·0. The most active preparations macerated discs in about 30 minutes at this pH. Dialysis which reduced the activity of the viscosity-reducing enzyme also reduced macerating activity and there was no increase in activity when autoclaved preparations were added. When solutions were heated, macerating and viscosity-reducing activities were lost at about the same rate. As far as comparisons were made, therefore, the properties of the two enzymes were similar.

Discussion

This work has shown that the pectic enzymes produced by *P. debaryanum* are substantially different from those produced by two other soft-rot organisms *B. cinerea* and *E. aroideae*, and are also different from those described from other organisms. The enzyme produced by *P. debaryanum* which reduces the viscosity of pectin solutions can hardly be described as a polygalacturonase in the sense in which this term was originally used because pectates are not degraded, and because there is only a very limited degradation of pectin even after prolonged treatment. In some respects the viscosity-reducing enzyme of *P. debaryanum* resembles that produced by *Neurospora* (Roboz, Barratt, and Tatum, 1952) and that contained in a commercial preparation and described by Seegmiller and Jansen (1952).

A further interesting feature of the *P. debaryanum* enzymes is that although they did not degrade solutions of sodium pectate at pH 8·0, they did macerate discs of potato-tuber tissue readily at this pH. Maceration is generally considered to involve degradation of the middle lamella which is thought to consist mainly of the calcium and magnesium salts of pectic acid. If this be so then it is difficult to understand why *P. debaryanum* extracts should so readily macerate plant tissue and yet be unable to degrade pectates *in vitro*. It would seem either that the middle lamella contains esterified pectic materials such as can be degraded by *P. debaryanum* enzymes, or that in these particular experiments maceration resulted from degradation of esterified pectic substances in the primary cell-wall, the middle lamella not being attacked.

The results obtained with *P. debaryanum* are difficult to reconcile with the view that protopectinase activity is a combination of polygalacturonase and pectinesterase activity (Kertesz, 1951, p. 335). The main evidence for this view has been that solutions with protopectinase activity always had polygalacturonase activity as well. But *P. debaryanum* preparations which macerate plant tissue have no polygalacturonase or pectinesterase activity. It has still to be shown that the enzyme which reduces the viscosity of pectin but not of pectate solutions is the enzyme causing maceration. No evidence was obtained which showed that they are different.

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Physiological Causes of Differences in Grain Yield between Varieties of Barley

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With ten Figures in the Text

ABSTRACT

In a field experiment on barley at Rothamsted with the high mean yield of 49 cwt. of grain per acre, the varieties Proctor and Herta produced 10–15 per cent. more grain than Plumage-Archer on plots that received no nitrogenous fertilizer. When nitrogen was applied the difference was increased to about 30 per cent., because the higher nitrogen supply caused the Plumage-Archer crop to lodge and did not increase its yield, while Proctor and Herta remained standing.

The three varieties did not differ in leaf-area index nor in net assimilation rate before ear emergence, so that all had the same total dry weight. After ear emergence, the leaf-area indices of Proctor and Plumage-Archer were nearly equal, but that of Herta was smaller. Assuming that the photosynthetic efficiency of the leaves continued to be the same in all varieties, the higher grain yields of Proctor and Herta cannot be attributed to greater production of dry matter by the leaves, either before or after ear emergence. A pot experiment on plants with shaded ears confirmed that the dry matter contributed to grain yield by unit leaf area was nearly equal in all the varieties.

The higher grain yield of Proctor and Herta than of Plumage-Archer must therefore have come from additional photosynthesis in parts of the plant other than the leaves, i.e. in the ears themselves. An attempt to demonstrate this directly in a pot experiment, by comparing the grain yields of plants with shaded or with unshaded ears, was unsuccessful because the varieties behaved differently in pots; Proctor and Herta produced only about 6 per cent. more grain yield than Plumage-Archer, and though the decrease in grain yield by shading the ears was slightly greater for Proctor and Herta, the differences were not significant.

The sum of ear sizes (estimated from length and breadth measurements) per m.^{2} in the field experiment was greater for Proctor and Herta than for Plumage-Archer. Also the distribution of dry matter between developing ears and shoots apparently differed with variety, so that at ear emergence the dry weight of ears per m.^{2} was greater in the two higher yielding varieties. The increased amount of photosynthetic tissue in the ears of Proctor and Herta, as measured by size or weight, may not wholly explain their greater dry-matter production; ears of Herta may also have a higher photosynthetic efficiency.

No differences in nutrient uptake that could account for the varietal differences in grain yield were found. Plumage-Archer absorbed more potassium, and Herta less phosphorus than the other varieties. About a quarter of the final content of nitrogen, and a third of the phosphorus, was absorbed after ear emergence, but the potassium content was nearly maximal at ear emergence and later decreased.

The pot experiment showed that, on the average of all varieties, 26 per cent. of the dry matter in the grain at harvest originated from photosynthesis in the ears, including 10 per cent. from the awns; 59 per cent. came from photosynthesis

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in the flag-leaf lamina and sheath and peduncle, and 15 per cent. from parts of the shoot below the flag leaf.

INTRODUCTION

AN obvious way of investigating the physiological determinants of the yield of a crop species is to compare by growth analysis varieties that differ widely in yield. An opportunity and material for such a comparison has been provided by the recent history of barley varieties in England.

Formerly, the two varieties Plumage-Archer and Spratt-Archer were widely grown because of the high malting quality of their grain, and for many years they together accounted for nearly all the English barley crop, but recently a number of higher-yielding barleys introduced from Scandinavia have steadily replaced Spratt-Archer and Plumage-Archer. In 1953 the variety Proctor, bred by Dr. G. D. H. Bell at the Plant Breeding Institute, Cambridge, to combine the good malting qualities of the older English barleys with higher yield, was released to farmers; in 1954 about 7 per cent. of the barley sown in England was Proctor and the fraction increased to 56 per cent. in 1955 (McPherson, 1956) and to 65 per cent. in 1956 (Wood, 1957). This rapid increase in the area sown with Proctor has been mainly at the expense of Spratt-Archer and Plumage-Archer.

In three years' trials by the National Institute of Agricultural Botany (Elliott, 1954) the yield of grain of Proctor was nearly equal to that of Herta and Rika, the heaviest-yielding Scandinavian varieties, and about 15 per cent. above that of the control variety Kenia, which yields about the same as Spratt-Archer and a little more than Plumage-Archer. Proctor has nearly as stiff straw as Herta and Rika, but all three varieties are more productive than Spratt-Archer and Plumage-Archer even in conditions that cause none of them to lodge. Proctor barley appears to satisfy the requirements of English maltsters and brewers as well as Spratt-Archer and Plumage-Archer did, and it also meets the farmer's need for higher yield comparable with that of Herta and Rika, which produce grain unsuitable for malting.

The object of the work described in this paper was to investigate the physiological cause of the higher grain yield of Proctor; and to find out whether it is the same as in the Scandinavian barleys. A knowledge of the growth changes responsible for the increased yield might be a guide in future breeding work or might suggest ways of improving the management of the crop in the field. The variety formerly grown on Rothamsted farm was Plumage-Archer, because it is better suited to the heavy soil than Spratt-Archer. Accordingly, Proctor was compared in a field experiment with Plumage-Archer and Herta, and in a pot-culture experiment Kenia was added to the three varieties tested in the field, because Proctor originated from the progeny of a Plumage-Archer × Kenia cross and Herta from a Kenia × Isaria cross. Both experiments also measured the response of the varieties to nitrogenous fertilizer, as nitrogen supply is the most important nutrient factor affecting yield and malting quality.

In the field experiment, the progress of dry-matter accumulation and its distribution between different organs of the plant was followed by taking samples from the crops at regular intervals. Leaf-area determinations at the same times measured changes in the size of the photosynthetic system, and its efficiency was assessed by calculating net assimilation rates from dry weight and leaf area in the usual way. Plant and shoot numbers per unit area of crop and, later, ear numbers were counted, and ear size, number of grains per ear, and weight per grain were determined to relate yield to morphological characters. The samples were analysed for nitrogen, phosphorus, and potassium to find out whether varietal differences in yield depended on differences in nutrient uptake.

The dry matter that enters barley grain is derived from photosynthesis after the ears emerge (Archbold, 1942). Some of it is produced by photosynthesis in the ears themselves as well as in the parts of leaves and stems that remain green. The object of the pot experiment was to test whether the varieties differ in relative contributions made by ears, awns, leaves, and stems to grain yield.

MATERIAL AND METHODS

Field Experiment

Treatments: all combinations of

(*a*) three varieties: Plumage-Archer (A), Proctor (P), Herta (H);
(*b*) 0 *v.* 3 cwt. 'Nitrochalk' (0·46 cwt. N) per acre $\equiv$ 5·8 g. N per m.^{2} (N).

The treatments were arranged in a 6×6 Latin square with plots of approximately 1/45th acre (90 m.^{2}) on a site in Great Field 1, Rothamsted, that had been cropped with potatoes in 1954. The 'Nitrochalk' was broadcast on the seed-bed and every plot received a basal dressing of 1·15 cwt. per acre of a compound fertilizer containing 13 per cent. P_2O_5 and 13 per cent. K_2O. Each plot had 34 drill rows spaced 6 in. apart, of which 19 were harvested by combine-harvester and 10 were reserved for sampling at intervals during growth. The remaining rows served as guards at the edges of the plot and between the combine-harvested and sampled areas.

The experiment was sown on 22 March 1955 with a self-propelled seed and fertilizer drill constructed by the National Institute of Agricultural Engineering, each coulter of which delivers a separately measured volume of seed over a predetermined length of run. As the three varieties differed in grain size, three different measures were used, one for each variety, adjusted to contain the same number of grains. The rates of sowing were: Plumage-Archer 140 lb. (2½ bu.) per acre; Proctor 120 lb. per acre; Herta 125 lb. per acre (respectively 157, 135 and 140 kg. per ha.). The seed was dressed with a combined mercurial and insecticidal seed-dressing before sowing.

An infestation of *Polygonum persicaria* seedlings was controlled by spraying with DNOC on 12 May, immediately after the first sampling. The discoloration of the crop by the spray had almost disappeared by the second sampling on 25 May, and no sign of injury was apparent later.

Sampling procedure

On eight occasions at fortnightly intervals from 11 May to 17 August samples of the crop were taken from every plot. A sample consisted of a strip ½ m. wide running across 10 rows, located at random within the length of the plot and separated from previously sampled areas by an unsampled strip. Two samples per plot were taken on each occasion, representing together 10 m. of row or 1·52 m.^{2}

The plants were pulled up and the roots that remained attached were shaken as free from soil as possible. To save labour the number of shoots, and of ears after emergence, were counted in only half of each sample; the 5 rows nearest the edge of the plot were used for the counts in one of the samples and the other 5 rows in the second sample, so that all 10 sampled rows contributed equally to the counts. The number of plants as well as of shoots was counted on 11 May, but not subsequently. The whole sample was weighed, a 100 g. sub-sample was taken and, after the roots had been washed free of soil, dried at 100° C. From the dry-matter content of the sub-sample the total dry weight of the sample was calculated.

After ear emergence, ears and shoots in the counted half of each sample were separated and weighed, and a sub-sample of each was dried. The dry weights of ears and shoots per sample and their sum, the total dry weight per sample, were then calculated from the total fresh weight of the whole sample, using the fresh-weight ratio of ears: shoots and percentage dry-matter contents of ears and shoots determined on the separated half-sample.

The same procedure was followed at the last sampling, except that the half-samples not separated into ears and shoots were threshed to give yields of grain for comparison with those obtained from full-scale harvest by combine. Ears were counted in both halves of the sample.

The pairs of samples of dried material from each plot were bulked at every sampling, ground and used for estimations of nitrogen by the micro-Kjeldahl method, of phosphorus by a colorimetric method using stannous chloride and acid molybdate, and of potassium with a flame-photometer.

Measurements of leaf area

Leaf area per sample was estimated by a rating method, except on the first sampling occasion when the plants were too small and too uniform. At this time one plant from each sample was weighed separately, blue-prints were made of the leaves and their areas measured with a planimeter. The leaf area: plant fresh-weight ratio for each plant and the regression of the ratio on plant weight were calculated. From these and the total fresh weight and number of plants per sample the total leaf area per sample was determined by the method of Watson (1937).

At all subsequent samplings a modification of the rating method previously described for wheat (Thorne and Watson, 1955) was used. On each sampling occasion, a set of standard shoots representing a scale of distinguishable shoot sizes over the whole range to be found on the plots was collected and mounted on a board. Twenty shoots per sample, selected at random, were rated on this scale and the leaf areas of two (only one before 22 June) were measured. The leaf area corresponding to each class of the rating scale was obtained from the regression of log leaf area on class number calculated from the measured shoots. When separate regressions were calculated for each treatment on each occasion, the coefficients were found to vary with time but were independent of nitrogen supply or variety. Thus, there was no evidence that the operation of rating a shoot was influenced by variety or nitrogen treatment, and so the regression calculated over all varieties and treatments at each sampling time was used to define the leaf area of each class. Mean leaf area per shoot was calculated from the areas of the 20 rated shoots of a sample, and when multiplied by the number of shoots in the sample gave the total leaf area of the sample, which was expressed as leaf area index (L, leaf area per unit area of land).

At all samplings after the first, the leaf sheaths represented an appreciable fraction of the total green surface of the shoot. After ear emergence, photosynthesis in leaf sheaths and stem as well as in leaf laminae makes an important contribution to the yield of grain (Archbold, 1942), so it was decided to include them in the estimates of leaf area per shoot used in calibrating the rating scale. The length of each shoot and its diameters at bottom and top (auricle of the highest leaf before ear emergence; collar of the ear after ear emergence) were measured, and used to calculate its surface area. Before ear emergence the leaf sheaths overlap and cover all or nearly all of the shoot surface, but after ear emergence exposed parts of the stem and the peduncle account for some of the measured area. When the shoots began to ripen, only the green portions of leaves and stem were measured. The area of each leaf lamina was estimated by measuring its length and greatest breadth and multiplying their product by a conversion factor. To evaluate the factor and determine whether it varied with variety, nitrogen supply or stage of growth, 20 leaves for each treatment combination were selected at random on 27 May and on 7 July, blue-printed, and their areas measured with a planimeter. The conversion factors were estimated as the coefficients of regressions of measured area on length × breadth, calculated for each set of 20 leaves, the regression lines being constrained to pass through the origin. An analysis of variance of the coefficients showed no significant variation with treatment or sampling time, and the mean coefficient calculated for all the measured leaves, $0{\cdot}734 \pm 0{\cdot}0038$, was therefore used for all the data. The total leaf area of a shoot was taken as the sum of the areas of laminae and the surface area of the shoot.

Estimation of ear size

Photosynthesis in the ear, including the awns, supplies some of the dry matter that passes into the grains (Watson and Norman, 1939; Porter, Pal, and Martin, 1950), so varietal differences in ear size could affect grain yield. The following ear dimensions were measured on 10 randomly selected ears from each sample taken on 20 July: length from the collar to the tip of the apical spikelet (excluding awn); greatest breadth (excluding awns); length from the collar to the tip of the longest awn; number of fertile spikelets on one side of the ear. From these measurements the product of length × breadth per ear excluding awns was calculated, and the total length of awns per ear estimated.

Calculation of net assimilation rate

The mean net assimilation rate for each interval between samplings up to the time of ear emergence was calculated in the conventional way (Williams, 1946) for each plot from the total dry weights and total leaf areas of the samples taken at the beginning and end of the interval.

Pot Experiment

Treatments: all combinations of

(*a*) four varieties: Plumage-Archer (A), Proctor (P), Herta (H), and Kenia (K).

(*b*) 0 *v.* 1·5 g. ammonium nitrate (0·53 g. N) per pot (N).

Seed dressed with a mercurial seed dressing supplied by the National Institute of Agricultural Botany was sown on 17 March 1955 in glazed pots each containing 10 kg. of a mixture of a fertile Rothamsted soil with 20 per cent. sand to which 1·5 g. of K_2HPO_4 had been added. After germination the seedlings were thinned to 8 per pot, and the NH_4NO_3 added in solution to the pots of treatment N. Four replicates of each treatment were harvested on 18 May and 1 June before ear emergence.

As the ears emerged, the following treatments were applied in all combinations with (*a*) and (*b*): intact plants, not shaded (C) *v.* ears shaded (E) *v.* lower part of stems shaded (S), *v.* awns cut off (D). Four replicates of treatments C and E were harvested on 15 and 29 June and 13 and 27 July, and of treatments S and D on the last date only. The pots were arranged in the glasshouse in four randomized blocks, each containing a replicate of all combinations of treatment × sampling occasion (88 pots per block).

For treatment E, each ear was covered with an opaque shade when the basal spikelet became free from the flag leaf sheath. The shades consisted of cylinders, 14 cm. long × 2 cm. diameter, made of black light-proof paper covered with an outer layer of white paper. They were folded and fastened with a staple at the top, supported by a string from a cane placed centrally in each pot, and loosely closed around the peduncle at the base with a paper clip. For treatment S, all the stems in a pot were enclosed in a cylinder of black paper, closed at the top and provided with slits through which the stems projected, exposing to the light all of the stem above the base of the flag leaf sheath. For treatment D, the awns of treated ears were cut off close to the tip of each spikelet immediately after the ears emerged.

Sampling procedure

At each harvest fresh and dry weights per pot of living leaf laminae, stems including leaf sheaths, dead leaves and stems, and ears were determined. At the harvest on 15 June, when ear emergence had just started, ear weight included ears still enclosed in the flag leaf sheath, as well as those fully emerged. At the final harvest after the ears had been dried the grain was rubbed out, separated from the chaff, dried again, and weighed. The roots were washed free from soil with a jet of water, dried and weighed.

Leaf area per pot was estimated by measuring length and greatest breadth of the leaf laminae and the length and top and bottom diameters of the green stems, as in the field experiment. Every shoot was measured on 18 May but subsequently only those on two plants per pot. The total area of laminae per pot was calculated by multiplying the total fresh weight of laminae by the ratio of lamina area: lamina fresh weight of the two measured plants, and similarly for the total area of leaf sheaths.

The factor for converting length × breadth of lamina to area, determined on sets of 20 leaves of each treatment combination on 25 May and 24 June, was found to be independent of nitrogen supply, variety and time, and the mean value calculated from a regression for all the measured leaves was $0{\cdot}764 \pm 0{\cdot}0044$.

Net assimilation rates were calculated for each sampling interval from initial and final values of dry weight and leaf area obtained from similarly treated pots in the same block.

RESULTS

Field Experiment

General observations on growth

The plant populations were fairly variable in spite of the steps taken to secure uniform sowing; the number of plants counted in 10 m. of row per plot on 11 May had a standard error of 15 per cent. per plot. The treatment means (Table 1) showed no significant effect of nitrogen or variety. The mean plant number, 289 per $m.^2$, was 89 per cent. of the number of seeds sown.

TABLE 1

Field Experiment. Number of Plants per $m.^2$ on 11 May

	Proctor	Herta	Plumage-Archer	Mean
No N	287	269	290	282
N	272	298 (±18)	315	295 (±10)
Mean (±13) . .	279	283	303	289

Ear emergence occurred at the beginning of July between samplings 4 and 5. Herta was nearly a week earlier than the other varieties and some of its ears were beginning to emerge on 22 June. There was no observable difference between Proctor and Plumage-Archer in time of ear emergence.

Early in July Plumage-Archer began to lodge on the plots that received nitrogenous fertilizer. Eye-estimates of the percentage area of each plot lodged on 4 August gave the following means: Plumage-Archer with nitrogen 50 per cent., without nitrogen 8 per cent.; Proctor with nitrogen 5 per cent.; the rest were all standing.

There were no visible differences between varieties or nitrogen treatments in time of ripening, although the dry-matter content per cent. of fresh weight of the ears was greater for Herta and less for Plumage-Archer than for Proctor at both samplings 6 and 7. All the crops were fully ripe by the last sampling on 17–18 August, and at this time the dry-matter contents of ears and of grain were the same for all varieties.

Yield at final harvest

The yields of grain obtained by combine-harvesting the larger part of each plot (Table 2) were exceptionally heavy, often exceeding 50 cwt. per acre. Proctor gave a slightly higher yield than Herta in the absence of nitrogenous fertilizer, but responded less to nitrogen, so that the difference was reversed on the plots that received nitrogenous fertilizer, and on the average of both rates of nitrogen supply the two varieties gave identical yields. Plumage-Archer produced less grain than the other varieties at both rates of nitrogen supply, and as its yield was significantly decreased by nitrogenous fertilizer, presumably because it suffered severe lodging, the superiority of Proctor and Herta was much greater where nitrogen was given. In absence of nitrogenous fertilizer the mean of the Herta and Proctor yields was 13 per cent. greater than that of Plumage-Archer, but in presence of nitrogenous fertilizer it was 33 per cent. greater.

TABLE 2

Field Experiment. Yield of Grain (85 per cent. Dry Matter), cwt./acre, from Combine Harvest

	Proctor	Herta	Plumage-Archer	Mean
No N	51·0	48·5	43·9	47·8
N	52·7	55·6	40·6	49·6
		(±1·0)		(±0·6)
Mean (±0·7)	51·8	52·0	42·3	48·7
Difference (±1·4) . . .	+1·7	+7·1	−3·3	+1·8(±0·8)

The yields of grain estimated from the last sampling agreed well with those from the full-scale harvest by combine, though they were much less accurately determined. The mean yields for all treatments were: combine harvest 48·7±0·41 cwt. per acre, sampling harvest 48·2±1·23 cwt. per acre. The sampling harvest gave slightly larger estimates of the nitrogen responses than the combine harvest, but the differences between varieties were similar.

The combine harvest gave only grain yields, but the last sampling also provided estimates of yield of other parts of the crop. These are given as dry weights in Table 3 and, in common with other measurements on the samples, expressed per m.^{2} of crop.

The dry weight of ears showed differences between varieties similar to those in grain yield (Table 3) but the response to nitrogen of ear weight of Herta was apparently less than that of Proctor, the reverse of the result for grain yield. The lack of agreement between the nitrogen responses of grain yield and ear weight may be attributed to sampling errors, as they were estimated from different half-samples. The differences in nitrogen response between varieties were not significant, but those of grain yield were probably nearer to the true values because they agreed with the results of the large-scale harvest. The dry-weight yield of shoots (including some root) was less for Herta than the other varieties (Table 3). The difference between Proctor and Plumage-Archer was small. The total dry-weight of Proctor was therefore greater than that of Plumage-Archer, but Herta and Plumage-Archer gave almost identical yields of total dry weight. For Proctor and Herta the dry weight of shoots was less than that of ears, but Plumage-Archer had a greater weight of shoots than of ears. The variety × nitrogen interactions for shoot and total dry weights were not significant, but Proctor and Herta apparently responded more to nitrogen than Plumage-Archer, and the superiority of Proctor in total dry-weight yield was restricted to the plots that received nitrogenous fertilizer.

TABLE 3

Field Experiment. Data from Last Sampling Harvest, 17–18 August

	Mean of nitrogen treatments				Effect of nitrogen			
	Proctor	Herta	Plumage-Archer	S.E.	Proctor	Herta	Plumage-Archer	S.E.
Dry weight, g. per $m.^2$								
Grain	569	528	444	23	61	98	5	45
Ears	651	577	496	25	109	46	6	51
Shoots	601	527	623	21	144	107	61	42
Total	1,252	1,103	1,119	39	253	152	67	79
Number of ears per $m.^2$ . . .	744	725	542	22	106	57	27	43
Number of grains per ear . . .	21·0	19·1	20·6	0·46	1·1	2·3	1·0	0·92
Dry weight of 1,000 grains, g. . .	35·1	38·7	39·7	0·61	−2·9	0·2	−4·0	1·22

Proctor and Herta had more ears at harvest than Plumage-Archer (Table 3), and this accounted for their higher yield of grain, as they did not have more grains per ear or larger grains; on the contrary, Herta had fewer grains per ear than the others and Proctor had a smaller 1,000-grain weight. Because of these differences Plumage-Archer had a higher mean yield of grain per ear than either Proctor or Herta, and this partly compensated for its smaller number of ears. Nitrogenous fertilizer increased the number of ears, though only slightly for Plumage-Archer. It had little effect on the number of grains per ear of Proctor and Plumage-Archer and depressed their 1,000-grain weights, but it increased the number of grains per ear of Herta and did not affect its 1,000-grain weight, and these differences account for the greater response to nitrogen of Herta in yield of grain.

Analysis of growth

The fortnightly measurements of dry weight, leaf area and shoot number rarely showed significant interactions between nitrogen and variety, and accordingly only means for the three varieties, averaging the two rates of N supply, and the mean response to N for all varieties are given in Figs. 1–6. Any significant variety × N interactions are mentioned in the text.

Dry weight (Fig. 1)

Total dry-weight yield continued to increase until sampling 7, 2 weeks before harvest. There was no difference between varieties until sampling 5,

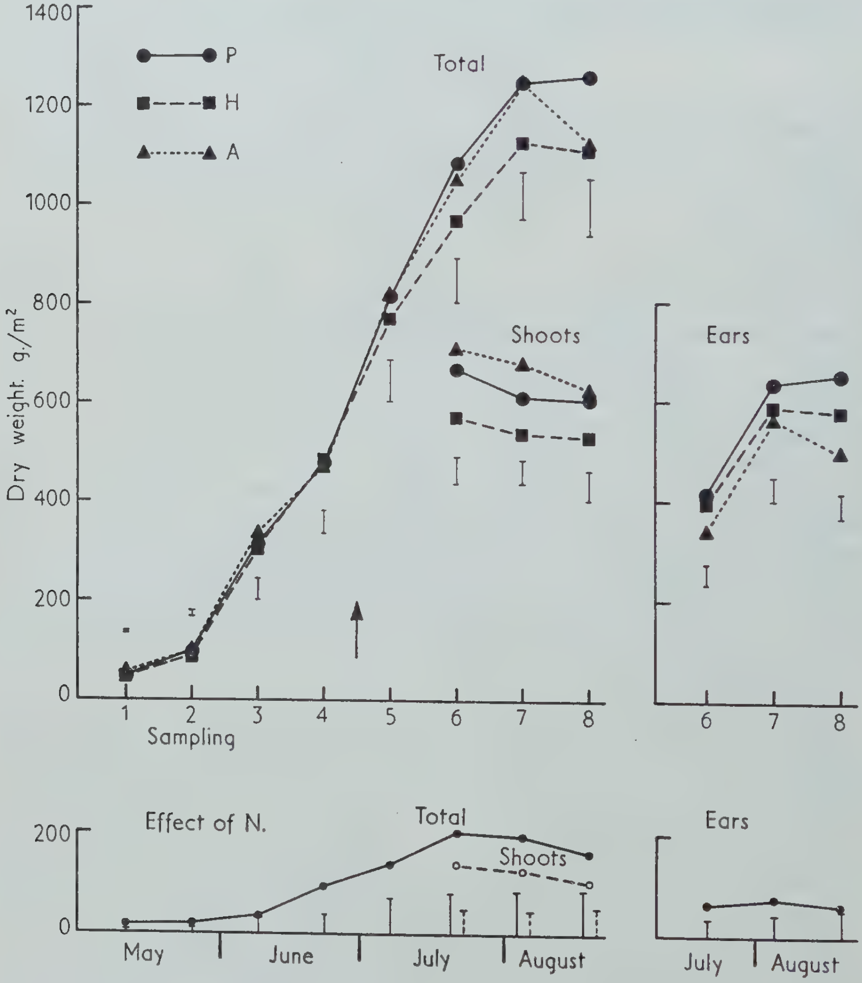

FIG. 1. *Field experiment.* Growth curves of total dry weight, and of the dry weights of ears and shoots of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments, and the change with time in the effect of nitrogenous fertilizer on dry weight, mean of all varieties. Vertical lines in this and subsequent figures represent least significant differences ($P = 0{\cdot}05$) and the arrow indicates approximately the mean date of ear emergence.

but subsequently Herta diverged from the other two varieties, and had a lower total dry weight at samplings 6 and 7. In the last interval, the total dry weight of Proctor and Herta did not change detectably, but that of Plumage-Archer decreased, so that at the final harvest Proctor had a greater total dry weight than the other two varieties.

After ear emergence, all the increase in dry weight occurred in the ears. The dry weight of shoots decreased a little with time and was consistently greater for Plumage-Archer than Herta, with Proctor intermediate. The dry weight of ears increased between samplings 6 and 7, and then remained nearly unchanged for Proctor and Herta, but apparently decreased for Plumage-Archer. The dry weight of ears of Plumage-Archer was always less than that of Proctor or Herta, and the differences widened at the last sampling.

Nitrogenous fertilizer increased total dry weight before ear emergence. The response increased steadily as the yield increased. On 22 June (sampling 4) it was significantly greater for Herta than the other varieties, but this interaction was not evident earlier or later. After ear emergence nitrogen increased the dry-weight yield of both shoots and ears. The effect on ear dry weight was less for Plumage-Archer than for the other varieties at sampling 7, and at the last sampling it fell to zero in Plumage-Archer but persisted in Herta and Proctor.

Leaf-area index (Fig. 2)

The leaf-area index L (including the green area of leaf sheaths and stems as well as leaf laminae) increased to a maximum of about 9 in early July (sampling 5) just after ear emergence, and then decreased rapidly to zero before sampling 8. The percentage of L ascribable to leaf laminae was independent of variety and nitrogen, and its mean varied with time as follows:

Sampling:	1	2	3	4	5	6	7
Lamina area as per cent. of L	100	79	71	57	38	23	6

The surface area of peduncle represented not more than 3 per cent. of L at samplings 5, 6, and 7. If leaf sheaths and stems were excluded from the leaf-area measurements, as in previous growth studies on cereals (Watson, 1952), L based on lamina area alone would show an earlier peak, at sampling 4, and its maximum value would be about 4·5.

L was the same for all varieties until sampling 4, but Plumage-Archer had a higher maximum than Herta at sampling 5, with Proctor intermediate. The differences between varieties later disappeared. Nitrogenous fertilizer greatly increased L at all times; at sampling 5 when L was maximal the response to N was about 30 per cent. of the mean.

Net assimilation rate (Table 4)

At no time were there any significant differences between varieties in net assimilation rate (E), and means for the three varieties over the period up to the completion of ear emergence (samplings 1 to 5) were almost identical. E was slightly decreased by nitrogenous fertilizer. The values of E were smaller than those previously found for barley in mid-summer (Watson, 1952)

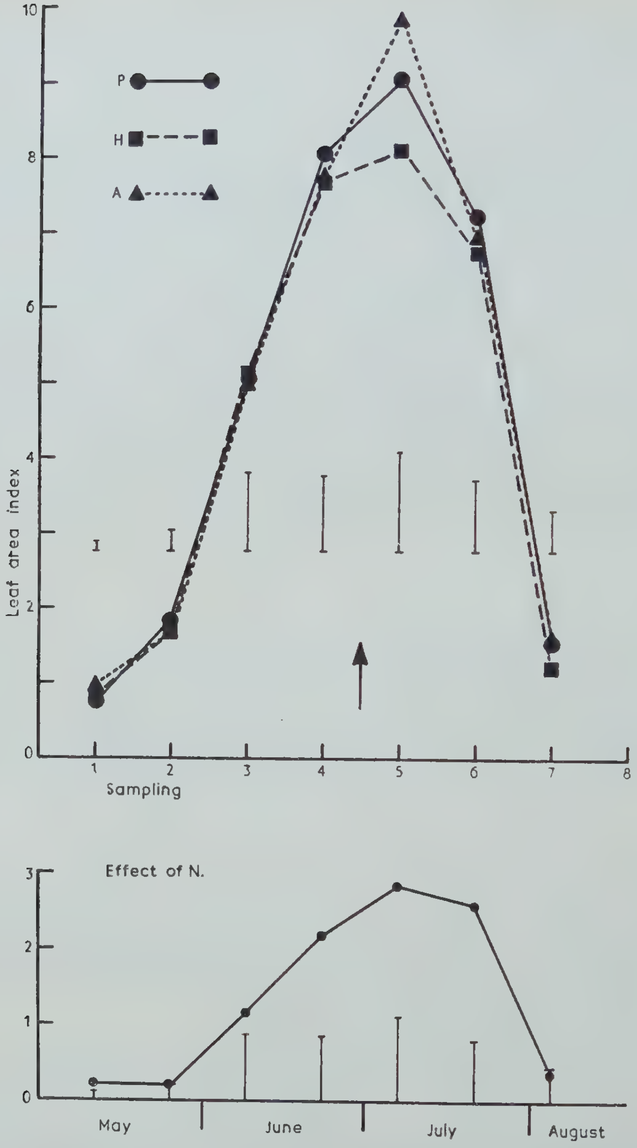

FIG. 2. *Field experiment.* Change with time in leaf-area index of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments, and in the effect of nitrogenous fertilizer on leaf-area index, mean of all varieties.

because inclusion of the leaf sheaths approximately doubled the estimates of leaf-area index on which E was based in the interval between samplings 3 and 5 in June and early July. Estimates of E for the intervals between samplings 5 and 7 during and after ear emergence have been omitted from Table 4 because their interpretation is complicated by the fact that the ears photosynthesize.

TABLE 4

Field Experiment. Net Assimilation Rates, g./m.²/week

	Interval between samplings				
	1–2	2–3	3–4	4–5	Mean
Mean of nitrogen treatments					
Proctor	22	33	14	21	22·4
Herta	20	37	15	19	23·0
Plumage-Archer	19	38	12	20	22·4
S.E.	1·4	2·7	1·9	1·6	0·82
Mean of varieties					
No nitrogen	21	39	13	22	23·9
Nitrogen	19	33	15	18	21·4
S.E.	1·2	2·2	1·5	1·3	0·66

Shoot number and ear number (Fig. 3)

Tillering had already started by the first sampling on 11 May and Plumage-Archer then had more shoots than the other varieties. The number of living shoots per m.² of Plumage-Archer increased to a maximum at sampling 3 in early June, and subsequently decreased steadily until the end of July, but the shoot number of Proctor and Herta continued to increase until July and few shoots of these two varieties died until they all ripened in August. Consequently, from early July onwards, Proctor and Herta had many more shoots than Plumage-Archer; Proctor consistently had more than Herta.

Proctor and Herta produced more ears than Plumage-Archer, both because they had a higher maximum shoot number and because a larger proportion of their shoots developed ears:

	Proctor	*Herta*	*Plumage-Archer*
Final ear number as per cent. of maximum shoot number	87	91	69

The number of ears per m.² did not change significantly after sampling 6.

Nitrogenous fertilizer increased shoot number and ear number at all times. The effect on shoot number increased with time as mean shoot number increased, and in July, when Proctor and Herta had more shoots than Plumage-Archer, they were also affected more by nitrogen.

Leaf area per shoot (Fig. 4)

As Plumage-Archer had fewer shoots than Proctor or Herta in July, its greater total leaf area at this time (Fig. 2) indicates that its shoots individually had more leaf area than those of the other varieties. The mean leaf area per shoot was similar for all varieties until sampling 4, but afterwards it became greater for Plumage-Archer than the others (Fig. 4). The leaf areas per shoot of Proctor and Herta were nearly equal at all times, so the greater total leaf area of Proctor was due to its higher shoot number. Nitrogenous fertilizer increased mean leaf area per shoot from early June onwards, but the increase was relatively smaller than that in shoot number.

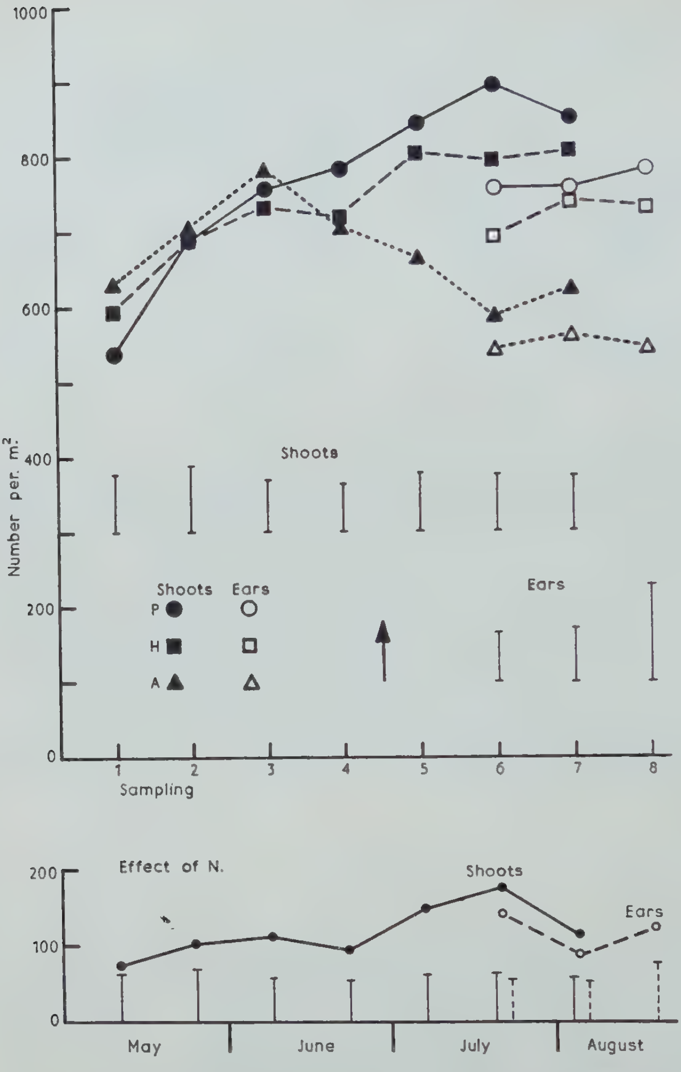

FIG. 3. *Field experiment.* Change with time in shoot number per m.² and number of ears per m.² of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments, and in the effect of nitrogenous fertilizer on shoot and ear number, mean of all varieties.

Ear size and length of awns (Table 5)

Both mean ear size, estimated as the product of length and breadth excluding awns, and the total length of awns per ear were greater for Plumage-Archer than the other varieties, and were nearly the same for Proctor and Herta. Nitrogenous fertilizer decreased the mean ear size of Plumage-Archer from 8·1 to 7·6 cm.², but had no other significant effects on ear size nor on awn length. The total ear surface, estimated as the sum of (length × breadth) for all ears per m.² of crop, and the total length of awns were both greater for

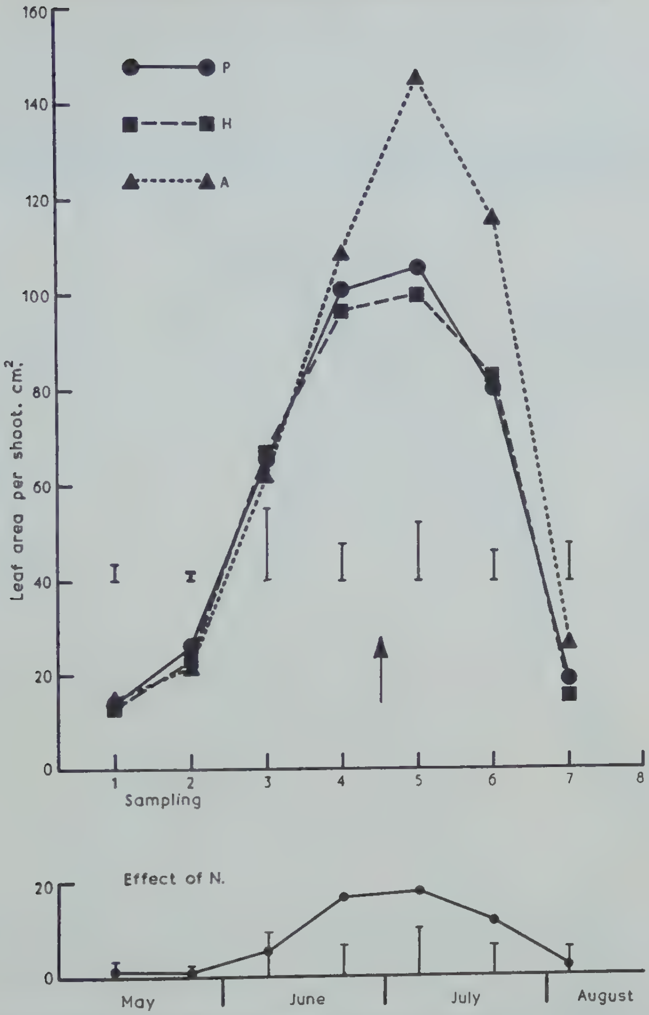

FIG. 4. *Field experiment.* Change with time in leaf area per shoot of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments, and in the effect of nitrogenous fertilizer on leaf area per shoot, mean of all varieties.

TABLE 5

Field Experiment. Estimates of Ear Size and Length of Awns; 20 July

(*Mean of nitrogen treatments*)

	Proctor	Herta	Plumage-Archer	S.E.
Mean product of length × breadth, cm.^{2} per ear	6·2	6·1	7·9	0·12
Sum of (length × breadth) for all ears, dm.^{2} per m.^{2}	47	43	43	1·3
Total length of awns, dm. per ear . .	33	32	41	0·8
Total length of awns, km. per m.^{2} . .	2·5	2·3	2·2	0·07

Proctor than the other varieties, but did not differ for Herta and Plumage-Archer.

Uptake of nutrients

Absorption of nitrogen and phosphorus continued until sampling 7 at the beginning of August (Figs. 5 and 6), but the total potassium content per m.^{2} of

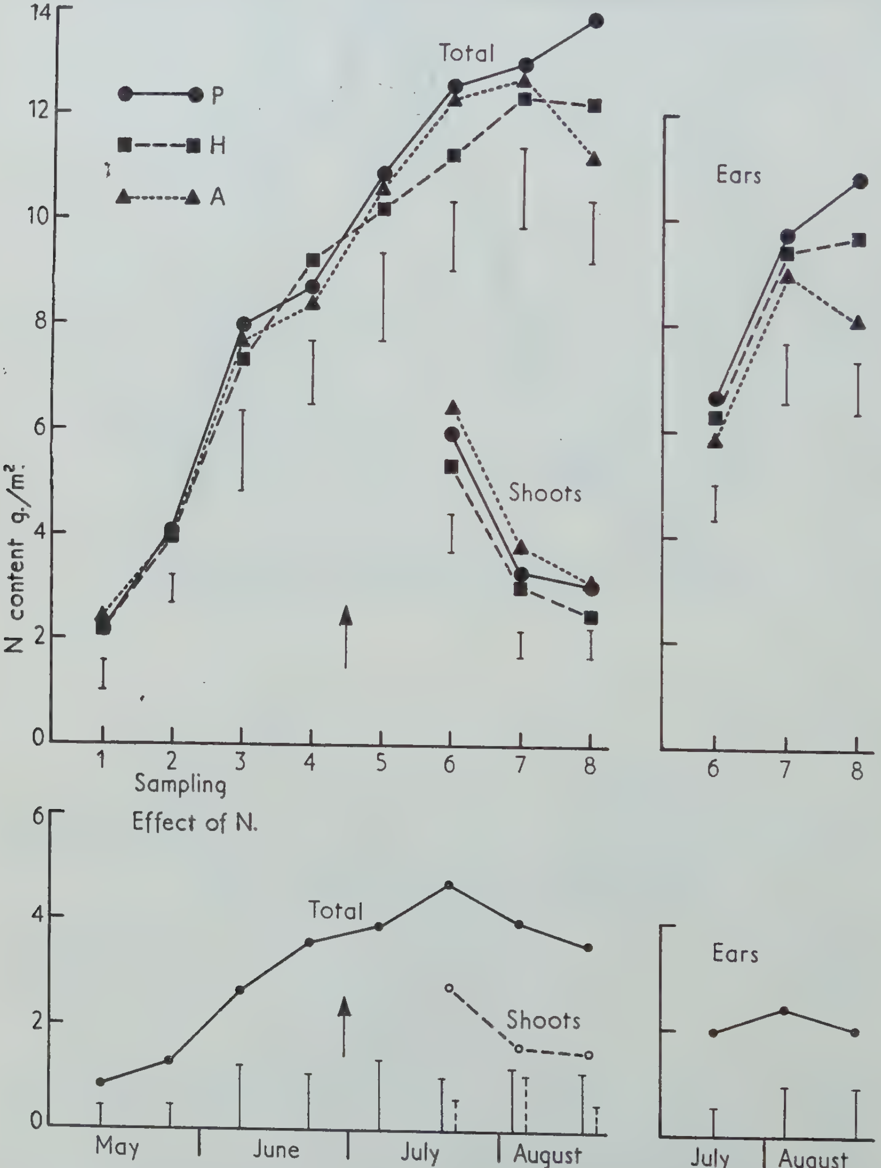

FIG. 5. *Field experiment.* Change with time in total nitrogen content, g./m.^{2}, and in the nitrogen content of ears and shoots of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments, and in the effect of nitrogenous fertilizer on nitrogen content, mean of all varieties.

crop reached its maximum at sampling 5 in early July, showed little change between samplings 5 and 7, and then sharply decreased (Fig. 7). This loss of potassium from the above-ground parts of ripening cereal crops is a well-known phenomenon. After ear emergence, nitrogen and phosphorus in the ear increased at the expense of the shoot, so that at the final harvest the major part of these nutrients occurred in the grain. Potassium in the ear increased only slightly after ear emergence, and most of the potassium remained in the shoot.

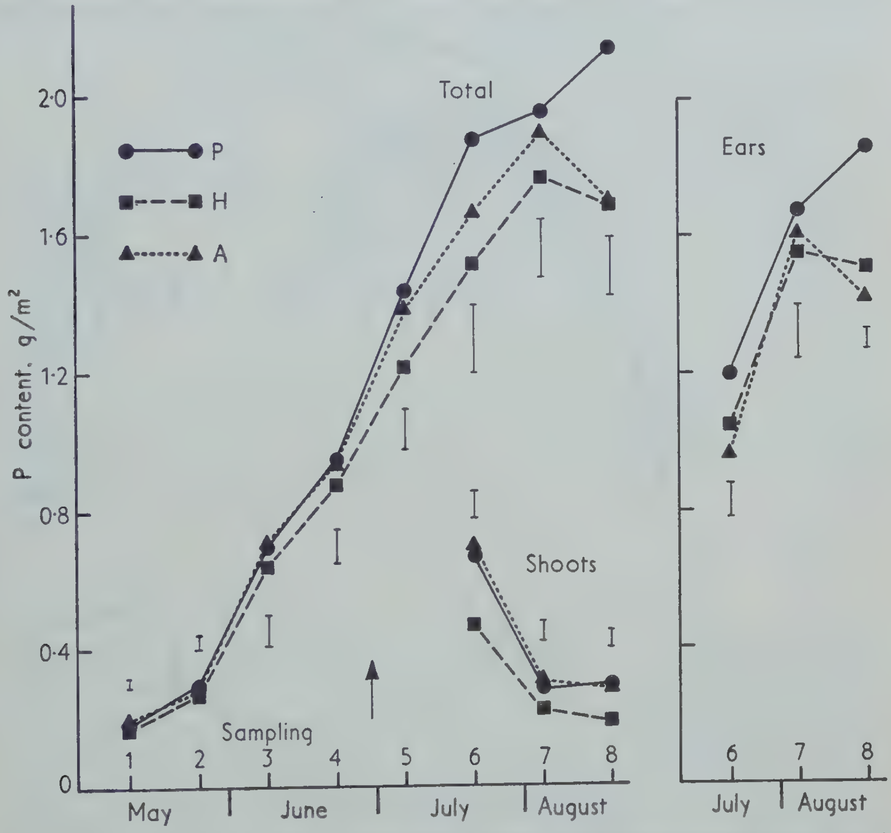

Fig. 6. *Field experiment.* Change with time in total phosphorus content, g./m.², and in the phosphorus content of ears and shoots of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments.

The differences between varieties in nutrient content before the final harvest were small. There were none in nitrogen content (Fig. 5); phosphorus content of Herta, both per m.² and per cent. of dry matter, was consistently lower than for the other varieties (Fig. 6); potassium content of Plumage-Archer in July and early August (samplings 5, 6, and 7), but not at other times, was higher than for the other varieties (Fig. 7) because Plumage-Archer had a higher potassium per cent. of dry weight of the shoot than Proctor and a greater dry weight than Herta. At the final harvest, the varietal differences in nutrient content of shoots, ears and grain (Figs. 5, 6, and 7; Table 6) mainly reflect differences in dry weight; nitrogen and potassium per cent. of dry matter were unaffected by variety, but phosphorus per cent. of dry matter of shoots, ears, and grain of Herta was slightly less than of the other varieties.

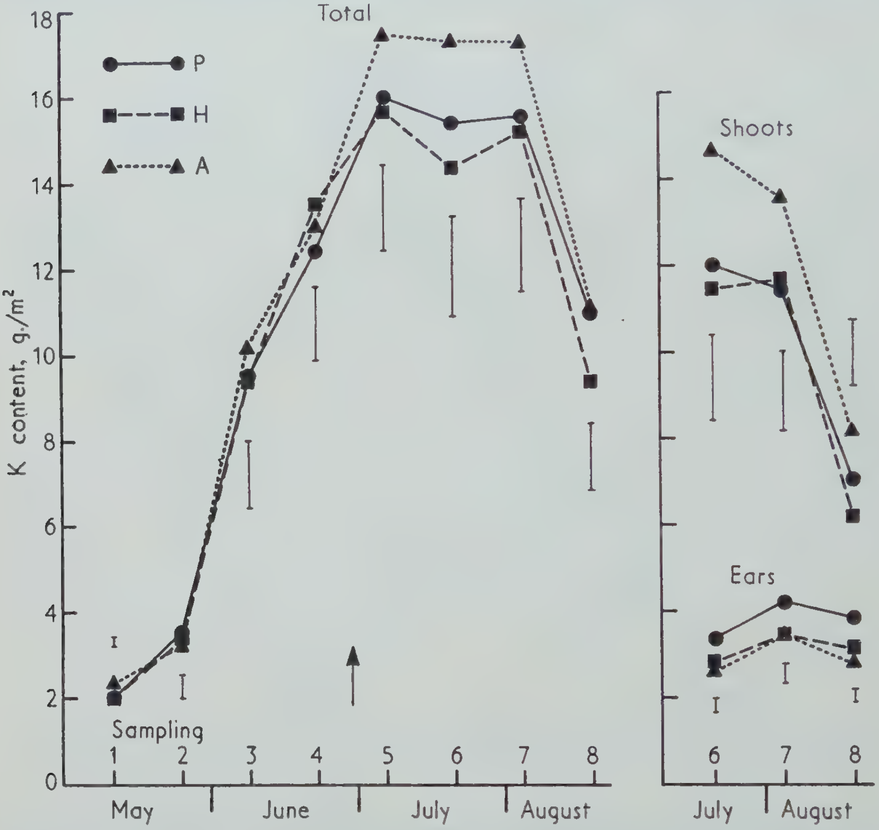

Fig. 7. *Field experiment.* Change with time in total potassium content, g./m.² and in the potassium content of ears and shoots of Proctor (P), Herta (H), and Plumage-Archer (A), mean of nitrogen treatments.

Nitrogenous fertilizer increased the nitrogen content of all parts of the plant and there was no significant interaction with variety until the last sampling when the increase for Proctor was greater than for Herta or Plumage-Archer. However, the increase in total N content of Proctor caused by nitrogenous fertilizer, 5·4±0·83 g. per m.², was anomalously high because it represents a recovery of over 95 per cent. of the added nitrogen. The mean recovery for the three varieties, 3·5±0·48 g. per m.², was 62 per cent. of the nitrogen in the fertilizer dressing. Previously, at harvest 7, the mean recovery was 68 per cent. and there were no significant differences between varieties. Nitrogenous fertilizer increased the uptake of phosphorus and potassium as well as of nitrogen. The increases were apparently larger, but not significantly, for Proctor than the other varieties.

TABLE 6

Field Experiment. Nitrogen, Phosphorus, and Potassium Contents of Grain at Last Sampling Harvest, 17–18 August

	Mean of nitrogen treatments			
	Proctor	Herta	Plumage-Archer	S.E.
Per cent. of dry matter				
Nitrogen	1·82	1·86	1·91	0·039
Phosphorus . . .	0·30	0·29	0·32	0·007
Potassium	0·51	0·49	0·49	0·010
g. per m.^{2}				
Nitrogen	10·4	9·9	8·4	0·41
Phosphorus . . .	1·7	1·5	1·4	0·07
Potassium	2·9	2·6	2·2	0·12

The nitrogen content per cent. of dry matter of the grain was much too high for malting purposes, even when no nitrogenous fertilizer was given, and the fertilizer application raised it from 1·7 to 2·0 per cent. There was no significant difference between varieties (Table 6).

Pot Experiment

General observations on growth

The mean dates of ear emergence, calculated from records of the number of ears shaded for treatment E each day during the period of emergence, were: Proctor 20 June, Herta 14 June, Kenia 15 June, Plumage-Archer 25 June, ±0·6 days. However, many ears of Plumage-Archer had partially emerged through the side of the leaf sheath for some days before they were covered, so these dates exaggerate the difference between Plumage-Archer and the other varieties in the time of emergence. Nitrogenous fertilizer hastened emergence by 1·6±0·4 days. Ears of Herta and Kenia which emerged earlier than those of the other varieties also ripened earlier. Proctor was the last to lose all green colour from the ears.

Yield at final harvest

The differences between varieties in yield of grain at the harvest on 27 July were relatively much smaller than in the field experiment. Averaging the two rates of nitrogen supply, Proctor and Herta had nearly equal yields, only about 6 per cent. greater than those of Kenia and Plumage-Archer (Table 7*a*), the difference between the means for the two pairs of varieties 2·0±1·1 g. per pot just failed to reach significance at the 5 per cent. level. Addition of nitrogen nearly doubled the grain yield, and the increase was greater for Herta than the other varieties, as in the field experiment, but Plumage-Archer was more responsive than in the field, and gave an increase intermediate between those of Herta and Proctor (Table 7*b*).

Plumage-Archer had a greater dry weight of chaff, shoots, and roots than the other varieties (Table 7*a*), and the increase in these parts produced by nitrogen also were greater for Plumage-Archer than for the others (Table 7*b*). Consequently, Plumage-Archer had a higher total dry weight than the others, and the difference was increased by application of nitrogen. The total dry weights of Proctor and Herta were nearly equal, but that of Kenia was a little less, particularly when nitrogen was given.

TABLE 7

Pot Experiment. Dry Weight, g. per pot at Final Harvest

Means of unshaded intact control plants (C)

	(*a*) Mean of nitrogen treatments					(*b*) Effect of nitrogen				
	Grain	Chaff	Shoots	Roots	Total	Grain	Chaff	Shoots	Roots	Total
Proctor	34·0	4·2	28·9	6·5	73·6	18·8	2·1	14·0	2·1	37·0
Herta	34·5	4·3	29·0	7·0	74·9	24·6	1·0	14·8	2·7	43·2
Kenia	32·0	4·2	27·2	7·4	70·8	15·5	1·7	11·7	2·9	31·9
Plumage-Archer	32·5	5·6	34·9	9·0	81·9	20·8	3·4	19·6	4·3	48·0
S.E.	1·11	0·25	0·95	0·47	2·29	2·22	0·50	1·91	0·94	5·58
Mean	33·2	4·6	30·0	7·5	75·3	19·9	2·0	15·0	3·0	40·0
S.E. of mean						1·11	0·25	0·95	0·47	2·29

Decrease caused by shading ears (C–E), *removing awns* (C–D), *or shading stems* (C–S)

(*c*) Mean of varieties and nitrogen treatments

	C–E	C–D	C–S	S.E.
Grain	8·6	3·3	4·9	0·78
Chaff	0·6	2·3*	0·4	0·18
Shoots	0·6	−0·5	0·3	0·67
Roots	0·4	−0·2	0·6	0·33
Total	10·3	4·9*	6·2	1·62

(*d*) Grain; mean of nitrogen treatments

	C–E	C–D	C–S
Proctor	8·7	2·0	4·8
Herta	10·9	5·8	5·8
Kenia	8·0	1·4	4·0
Plumage-Archer	7·0	4·1	5·1
S.E.		1·57	
Mean of varieties			
No N	5·9	1·5	2·9
N	11·4	5·1	7·0
S.E.		1·11	

* Includes dry weight of awns removed (2·1 g. per pot).

Shading the ears or lower parts of the stem, or cutting off awns, had almost negligible effects on the dry weight of parts of the plant other than the grain (Table 7*c*); shading ears slightly reduced the weight of chaff, but none of the other effects on weight of chaff, shoots or roots were significant.

On the average of all varieties and treatments shading ears decreased the yield of grain by 8·6 g. per pot, or 26 per cent. of the yield of unshaded plants (Table 7*c*). The loss of yield caused by shading ears did not vary significantly between varieties (Table 7*d*), but it appeared to be a little greater for Proctor and Herta; it was twice as great in plants that received nitrogen as in those that did not. The percentage loss of grain yield caused by shading the ears calculated from the means in Table 7 was slightly higher for Herta (32 per cent.), and lower for Plumage-Archer (21 per cent.) than for the others (Proctor 26 per cent., Kenia 25 per cent.). It was nearly independent of nitrogen supply (No N 25 per cent.; N 27 per cent.).

The mean decreases of grain yield caused by removing the awns or by shading the stems were respectively 3·3 and 4·9 g. per pot (10 and 15 per cent. of the yield of intact unshaded plants) (Table 7*c*). Neither of these effects varied significantly with variety, and both were greater in the plants that received nitrogen (Table 7*d*).

As in the field experiment, Plumage-Archer had fewer ears and a greater 1,000-grain weight than the other varieties, and more grains per ear than Herta and Kenia, but not Proctor (Table 8*a*). Nitrogenous fertilizer increased the number of ears per pot of Plumage-Archer and Herta more than of the others; it had no detectable effect on the number of grains per ear and 1,000-grain weight of Plumage-Archer, but increased them in the other varieties (Table 8*b*).

TABLE 8

Pot Experiment. Components of Grain Yield at Final Harvest

Means of unshaded, intact control plants (C)

	(*a*) Mean of nitrogen treatments			(*b*) Effect of nitrogen		
	No. of ears per pot	No. of grains per ear	Dry weight of 1,000 grains, g.	No. of ears per pot	No. of grains per ear	Dry weight of 1,000 grains, g.
Proctor	34·4	26·5	36·2	12·8	3·0	3·1
Herta	36·9	24·6	36·0	16·2	5·6	4·0
Kenia	34·9	24·8	36·3	9·8	4·3	1·4
Plumage-Archer	27·2	27·7	42·7	16·5	1·4	−0·8
S.E.	1·06	0·49	0·56	2·12	0·98	1·12
Mean	33·3	25·9	37·3	13·8	3·6	2·0
S.E. of Mean				1·06	0·49	0·56

Decrease caused by shading ears (C–E), *removing awns* (C–D) *or shading stems* (C–S)

(*c*) Mean of varieties and nitrogen treatments

	C−E	C−D	C−S	S.E.
Number of ears per pot	0·1	−0·2	0·7	0·75
Number of grains per ear	0·8	0·0	−0·7	0·35
Dry weight of 1,000 grains, g.	8·7	3·7	5·6	0·40

(*d*) Dry weight of 1,000 grains, g. Mean of nitrogen treatments

	C−E	C−D	C−S
Proctor	7·2	2·9	5·5
Herta	9·3	3·3	4·3
Kenia	9·0	4·0	5·0
Plumage-Archer	9·4	5·4	7·7
S.E.		0·79	

Shading ears or stems or removing awns decreased the yield of grain almost wholly by decreasing 1,000-grain weight (Table 8*c*). The only other significant effect was a small decrease in number of grains per ear by shading the ears. Shading ears apparently affected the 1,000-grain weight of Proctor less, and shading stems affected the 1,000-grain weight of Plumage-Archer more, than those of the other varieties (Table 8*d*).

Analysis of growth

Dry weight. The total dry weight per pot of intact unshaded Plumage-Archer plants began to exceed those of the other varieties in June (Fig. 8), and by July (sampling 5) it was significantly greater. Plumage-Archer consistently had a greater dry weight of roots than the other varieties and a greater shoot dry weight after ear emergence, but did not differ from the other varieties in dry weight of ears. Proctor, Herta, and Kenia had similar dry weights of ears, shoots, and roots.

Nitrogen greatly increased the dry weights of ears, shoots, and roots, but there were no significant interactions with variety.

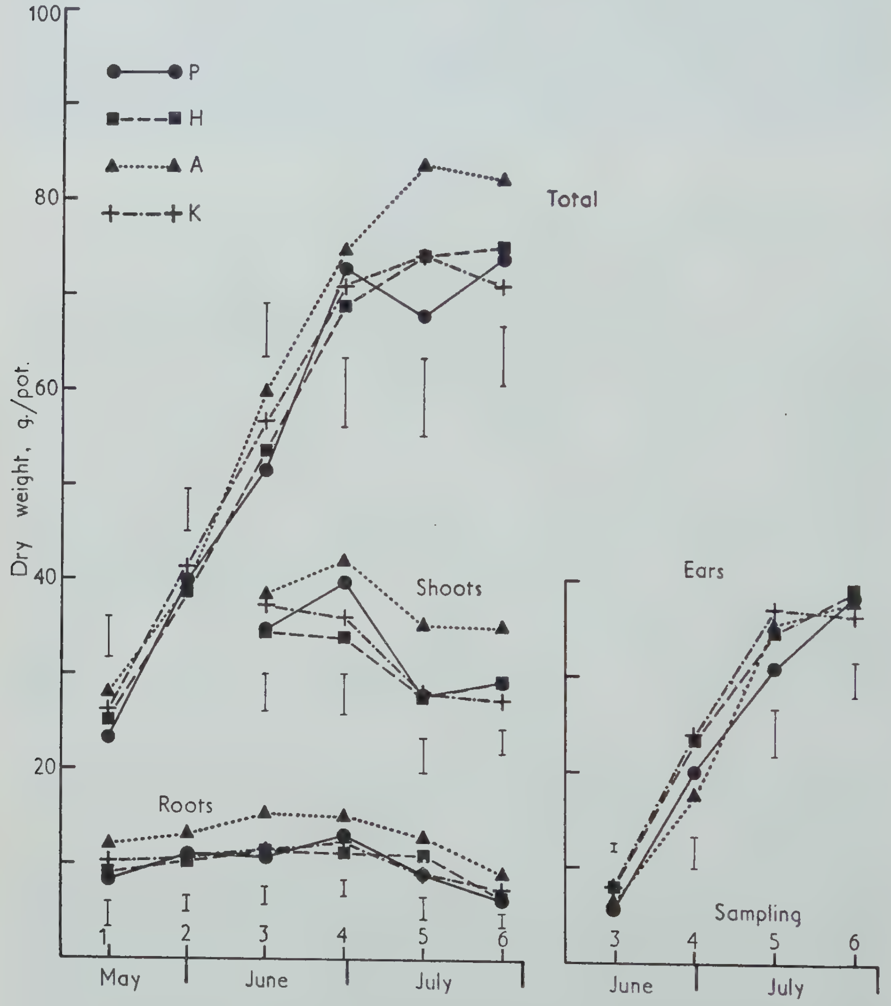

FIG. 8. *Pot experiment.* Growth curves of total dry weight, g. per pot, and of the dry weight of shoots and roots of intact, unshaded plants of Proctor (P), Herta (H), Plumage-Archer (A), and Kenia (K), mean of nitrogen treatments.

Leaf area. Leaf area (including green areas of sheaths and stems) (Fig. 9) reached a maximum in mid-June (sampling 3), and then decreased nearly to zero by mid-July. Near to the peak, Plumage-Archer had a greater leaf area than Kenia and Herta, with Proctor intermediate. Nitrogenous fertilizer almost doubled leaf area and did not affect the differences between varieties.

Shading the ears slightly delayed senescence of the leaves; on 13 July the mean leaf area per pot surviving on unshaded plants was 1·6 dm.^{2}, and for pots with shaded ears 4·1 dm.^{2} ($\pm$0·54). No measurements of leaf area were made on plants with shaded shoots, but a scoring for colour on 11 July showed that shading the lower part of the shoots, like shading the ears, slightly delayed the yellowing of leaves and stems.

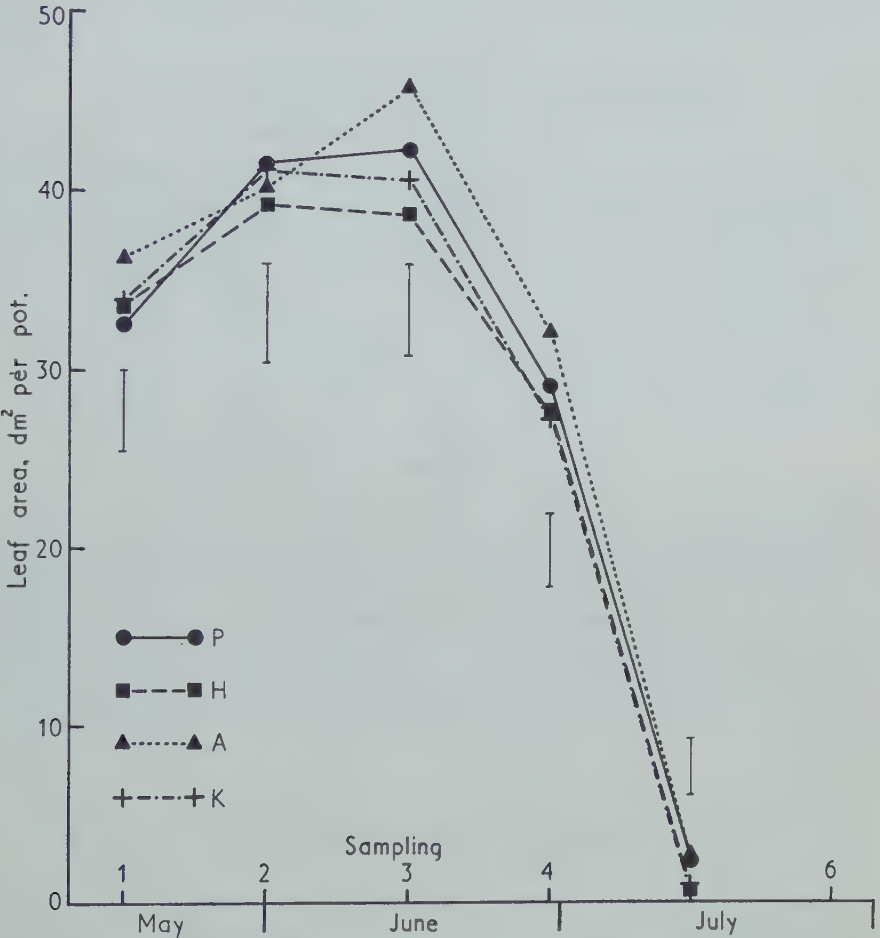

FIG. 9. *Pot experiment.* Change with time in leaf area, dm.^{2} per pot, of intact unshaded plants of Proctor (P), Herta (H), Plumage-Archer (A), and Kenia (K), mean of nitrogen treatments.

Net assimilation rate

Net assimilation rates were computed for the three intervals between the first four sampling times (Table 9). The values for the two later intervals refer only to plants with shaded ears, thus excluding any contribution by photosynthesis in the ears to the increments of dry matter. The only significant difference between varieties was that Plumage-Archer had a higher net assimilation rate than the others in the second interval, but this difference did not occur consistently; in the previous interval Plumage-Archer had the lowest net assimilation rate. The means for the whole period for Proctor, Herta, and Kenia were nearly identical, and that for Plumage-Archer was slightly, but not significantly, greater than the others. Net assimilation rate was consistently increased by nitrogenous fertilizer.

TABLE 9

Pot Experiment. Net Assimilation Rate, g./m.²/week

	Intervals between samplings			
	1–2	2–3*	3–4*	Mean
	Mean of nitrogen treatments			
Proctor	24	12	13	16·2
Herta	19	14	16	16·6
Kenia	20	12	17	16·6
Plumage-Archer	16	24	16	18·6
S.E.	2·4	3·2	4·5	1·05
	Mean of varieties			
No N	17	13	14	14·9
N	22	18	17	19·0
S.E.	1·7	2·2	3·2	0·74

* For plants with ears shaded.

Number of shoots

In April, when shoot number was still increasing, Plumage-Archer had more shoots, and Proctor less, than the other varieties (Table 10). At sampling 1 on 18 May, near to the time of maximum shoot number, the differences between varieties disappeared. Later, more shoots of Plumage-Archer died than of the other varieties. The effect of nitrogen was relatively small compared with those on dry weight and leaf area, and there was no nitrogen × variety interaction.

TABLE 10

Pot Experiment. Number of Shoots per pot

	21 April	18 May	15 June
	Mean of nitrogen treatments		
Proctor	28	45	35
Herta	35	47	34
Kenia	32	48	34
Plumage-Archer	38	47	29
S.E.	0·9	1·7	0·7
	Mean of varieties		
No N	32	44	26
N	35	50	40
S.E.	0·6	1·2	0·5

DISCUSSION

In the field experiment, Proctor and Herta showed the expected superiority to Plumage-Archer in yield of grain. On plots that received no nitrogenous fertilizer, the difference in yield (Table 2) was similar to that found in the National Institute of Agricultural Botany field trials, but it was much greater where nitrogen was given, amounting to 30 per cent. of the yield of Plumage-Archer. This large difference was no doubt partly due to lodging of the Plumage-Archer crop. The mean yields of grain in g./m.^{2} of samples from lodged and unlodged areas of Plumage-Archer at the final sampling harvest were:

	No N	N
Unlodged	446	514
Lodged	422	399

Comparison with Table 3 shows that if the Plumage-Archer crop had not lodged it would still have given the lowest yield at both rates of nitrogen supply, although the differences in yield would have been smaller; they might have been less than these figures indicate, because the heavier parts of a crop are more prone to lodge.

Up to the time of ear emergence the three varieties did not differ in yield of dry matter (Fig. 1). They had nearly identical leaf area indices and their net assimilation rates also were not detectably different. Therefore the higher grain yield of Proctor and Herta cannot be a consequence of greater dry-matter production before the ears emerged.

After ear emergence, the leaf-area indices of Proctor and Plumage-Archer continued to be nearly equal, while that of Herta was smaller, especially at sampling 5 when leaf area was maximal. Assuming that the photosynthetic efficiencies of the leaves continued to be similar for all varieties, as they were before ear emergence, the higher grain yield of Proctor and Herta than of Plumage-Archer cannot be attributed to greater dry-matter production by the leaves. It is unlikely that a larger fraction of the product of photosynthesis in the leaves and stems after ear emergence passed into the grain of Proctor and Herta, leaving a smaller fraction in the shoots, because the dry weight of shoots decreased in this period (Figs. 1 and 8) indicating that all the assimilate produced in the shoots was transferred to the ears. If any was retained in the shoots it was insufficient to offset losses, by respiration or export, of dry matter produced previously. It follows that the additional dry matter entering the grain of Proctor and Herta must have originated from photosynthesis after the time of ear emergence in some other part of the plant than the leaves, and there can be little doubt that the ears themselves were responsible.

Greater dry-matter production by the ears must depend on increase in either their photosynthetic capacity, or their photosynthetic efficiency, or both. Increase in the photosynthetic capacity of the ears (if this is to be measured by their weight) without change in the total dry weight of the crop at ear emergence, must involve a change in the distribution of dry matter, a larger fraction going to form ears and a smaller fraction the shoots. Fig. 1 suggests that there was such a difference between the varieties in dry-matter distribution; at the 6th and later samplings the dry weights of ears per m.^{2} of Proctor and Herta were greater, and the dry weights of shoots less, than those of Plumage-Archer. But the relative weights of ears and shoots at these times were affected by differences between varieties in growth of the grain since ear emergence; thus, even at sampling 6 the greater dry weight of ears of Proctor and Herta may already have been due partly to greater grain weight produced by increased photosynthesis in the ears, and that of Herta partly to the longer period of grain growth resulting from earlier ear emergence. However, the dry weights of shoots per m.^{2} were presumably free from these complications, and continued to reflect differences established at the time of ear emergence by variation in the partition of dry matter between ear and shoot. To confirm that varieties differ in the distribution of dry matter between shoot and developing ear, measurements need to be made on individual shoots as emergence begins, or at anthesis, so as to eliminate any effect of differences in rate of growth of the grain.

The greater number of ears per m.^{2} of Proctor and Herta cannot be regarded as the origin of the increased photosynthetic capacity of the ears, because it was accompanied by smaller mean ear weights that persisted until the final harvest:

Mean dry weight per ear, g.	Proctor	Herta	Plumage-Archer
Sampling 6 . . .	0·55	0·58	0·63
Final harvest . . .	0·84	0·80	0·92

Higher ear number may be correlated with the altered partition of dry matter between ear and shoot; a tendency for nutrient materials to move more readily into the developing ear might be expected to favour survival of a higher proportion of shoots to the stage of flowering and production of mature ears.

By analogy with leaf area, the projected area of an ear may be a better measure of photosynthetic capacity than its weight. Table 5 shows that mean ear size, estimated as the product of length and greatest breadth excluding awns, like ear weight, was less for Proctor and Herta than Plumage-Archer. The summation of ear sizes per m.^{2} (mean ear size × number of ears per m.^{2}) was greater for Proctor, but was the same for Herta and Plumage-Archer. Similar results were found for the total length of awns per m.^{2} On this basis, increased dry-matter production by the ears was associated with an increase in the photosynthetic capacity of the ears of Proctor but not Herta, and if this conclusion is correct it implies that ears of Herta had a higher photosynthetic efficiency than those of the other varieties.

At the final harvest the total dry weight of Proctor was greater than that of Plumage-Archer. This difference apparently developed only after sampling 7 (Fig. 1) by loss of dry matter from the Plumage-Archer plants, perhaps partly attributable to lodging. However, the apparent increase in shoot number of Plumage-Archer between samplings 6 and 7 (Fig. 3) seems improbable and suggests that the samples of Plumage-Archer taken at sampling 7 were erroneously large, and so may have overestimated the total dry weight. The divergence between Plumage-Archer and Proctor may, therefore, have begun before sampling 7 (there is an indication of it at sampling 6) and the difference at the final harvest was probably due partly to greater dry-matter production by Proctor, and not solely to subsequent loss by Plumage-Archer. The argument of the previous paragraphs implies that the additional dry matter was the result of more photosynthesis by the ears of Proctor, and not by the leaves. The leaf-area index of Herta after ear emergence was less than that of Plumage-Archer, and the consequent decrease in dry-matter production was evidently not fully offset by increased production in the ears, because the total dry weight of Herta was consistently less than that of Plumage-Archer.

Unfortunately, the varieties did not behave in the same way in pot-culture as in the field, for they did not differ significantly in grain yield and the results (Table 7*a*) indicate that Proctor and Herta produced only 6–7 per cent. more grain than Plumage-Archer. Also Plumage-Archer had a greater total dry weight (Fig. 8) and maximum leaf area (Fig. 9) than the other varieties. The same result was found in a subsequent pot experiment not reported here. However, the field and pot experiments showed similar varietal differences in the distribution of dry matter between the grain and the rest of the plant at harvest, as the following figures for grain as percentage of total dry weight show:

	Proctor	Herta	Plumage-Archer
Field experiment . .	44	44	36
Pot experiment . .	46	46	40

Also, in the pots as in the field, Plumage-Archer had fewer ears and larger grains than the other varieties. Apparently the conditions of pot culture were more favourable to Plumage-Archer than the other varieties, but did not change the pattern of development. The reason for the difference in the relative performance of the varieties between the field and the pots is not known.

The differences between varieties in loss of grain yield caused by shading the ears (Table 7*d*; C–E) were too small to be significant, but they agree with the conclusion from the field experiment that photosynthesis in the ears contributes more to the grain yield of Proctor and Herta than of Plumage-Archer. The difference between Proctor or Herta and Plumage-Archer in amount of photosynthesis in the ears would presumably be larger if the difference in total grain yield were relatively greater, as in the field experiment.

The grain yield of plants with shaded ears measured the contribution of photosynthesis in the shoots to the grain yield of unshaded plants. It is difficult to relate the size of this contribution in the different varieties to the photosynthetic area of the shoots after the time when the grains began to fill, because this time varied between varieties and was not accurately defined, though it was presumably near to ear emergence. For this purpose it is preferable to consider amounts of dry matter added to the grain in the same interval for all varieties, but as grain yield was determined only at the final harvest it was necessary to use the increment in ear dry weight as a measure of the increment in grain yield. The interval between sampling 3, near to the time of ear emergence, and the final harvest was selected. The increment in ear dry weight during this interval represents about 90 per cent. of the final grain yield; the discrepancy is partly due to inclusion of the adherent glumes in grain yield and partly to growth of some grains starting before sampling 3.

Table 11 shows that the mean increment after sampling 3 of ear dry weight of plants with shaded ears was greater for Proctor and Plumage-Archer than for the other varieties. The mean integrated leaf area over the interval (the leaf-area duration) also was greater for these two varieties, and consequently the ratio of dry-matter increment to leaf-area duration was nearly the same for all, that is to say, the leaves of the four varieties were equally efficient in producing dry matter to fill the grain. The ratio is an approximation to the mean net assimilation rate after ear emergence, and the results confirm the conclusion from measurements made in pot and field experiments before ear emergence that the varieties did not differ in net assimilation rate. Table 11 also shows that the dry matter contributed to grain yield by assimilation in the shoots was nearly doubled by the nitrogen treatment, and that the increase was wholly ascribable to increase in leaf-area duration, because the ratio of dry-matter increment of the ears to leaf-area duration was independent of nitrogen supply. This indicates that the photosynthetic efficiency of the leaves after ear emergence was unaffected by nitrogen, though previously it had been increased (Table 9).

TABLE 11

Pot experiment. Contribution of assimilation in shoots to grain yield, estimated as the increment in dry weight of ears, g. per pot, between sampling 3 and final harvest of plants with shaded ears (ΔW), and its relation to leaf area duration, dm.^{2} weeks, for the same period (D).

	ΔW	D	$\Delta W/D$
	Mean of nitrogen treatments		
Proctor	23·2	117	22·1
Herta	19·9	103	19·7
Kenia	20·6	98	21·1
Plumage-Archer	24·1	124	20·4
S.E.	1·1	3·8	1·2
	Mean of varieties		
No N	15·5	76	21·2
N	28·4	144	20·5
S.E.	0·8	2·7	0·8

The mean leaf-area duration after ear emergence in the field experiment, estimated by integrating leaf-area index in the interval between sampling 5 and the final harvest and averaging both rates of nitrogen supply, was not significantly different for the three varieties:

	Proctor	Herta	Plumage-Archer	S.E.
Mean leaf-area duration, weeks	26·6	23·9	26·9	1·1

Therefore, if the leaf area of all varieties was equally efficient in contributing dry matter to the grain, as in the pot experiment, the higher grain yields of Proctor and Herta than of Plumage-Archer cannot be attributed to a greater contribution from photosynthesis in the leaves. This supports the conclusion (p. 345) reached by assuming that the similarity of all the varieties in the field experiment in net assimilation rate before ear emergence persisted afterwards. The leaf-area duration for Herta was slightly smaller than for the other varieties, but this difference might disappear if allowance were made for the earlier emergence of its ears.

No difference between varieties in the contribution of photosynthesis in the awns to grain yield was established, although the variety means were somewhat variable (Table 7*d*). On the average of all varieties and nitrogen treatments, cutting off the awns reduced grain yield by 10 per cent.; this is similar to the results of other workers (Vervelde, 1946). Nor were there any varietal differences in the contribution of the part of the shoot below the base of the flag leaf sheath; the mean value was 15 per cent. of the grain yield. Porter, Pal, and Martin (1950) concluded that no carbohydrate migrates to the ear from this part of the plant, but shading experiments on wheat showed a small contribution from the lower leaves and stem (Boonstra, 1929). The results of the pot experiment showed that, on the average, the dry matter in the grain was derived from photosynthesis in different parts of the plants in the following proportions: 26 per cent. from the ears, of which 10 per cent. was contributed by the awns; 59 per cent. from the flag-leaf lamina and sheath and the peduncle; 15 per cent. from the parts of the shoot below the base of the flag leaf sheath. This agrees with earlier estimates of the extent of photosynthesis in the ears of barley (Watson and Norman, 1939), and clearly illustrates the overriding importance of the contribution from the flag leaf.

There were no differences between varieties in nutrient uptake that could explain the differences in yield. Plumage-Archer with a smaller yield of grain than the other varieties absorbed more potassium, while Herta, which yielded better than Plumage-Archer and about the same as Proctor, absorbed less phosphorus than either. Although the progress curves of nutrient uptake have little bearing on the problem of varietal difference in yield, they have considerable general interest because there is surprisingly little information on the course of nutrient uptake by cereal crops. It appears to be widely believed that nearly all mineral nutrients are absorbed in the early stages of growth, and that there is little further uptake after ear emergence. Thus, Gregory (1953) says that in the developing cereal plant over 90 per cent. of the total nitrogen taken up by the plant has been accumulated when the dry weight is only 25 per cent. of the final value, and that the same is true of phosphorus. Richardson and Trumble (1928) found for barley grown in pots that at ear emergence over 90 per cent. of the final contents of nitrogen and potassium and nearly 80 per cent. of the phosphorus had already been absorbed; in the experiment of Knowles and Watkin (1931) on a field crop of wheat the comparable figures were over 80 per cent. of the nitrogen and nearly 95 per cent. of the phosphorus, while the potassium content was maximal at ear emergence and subsequently decreased. For comparison with these results, Fig. 10 shows the average changes in total dry weight, and in the nitrogen, phosphorus, and potassium contents of the whole plant expressed as per cent. of their maximal values, in the field experiment. By sampling 3, when 25 per cent. of the final dry weight had been produced, only 60 per cent. of the maximal contents of nitrogen and potassium, and only 35 per cent. of the phosphorus, had been absorbed. About 75 per cent. of the nitrogen, 60 per cent. of the phosphorus, and nearly all the potassium eventually absorbed were present in the plants at ear emergence. The results were similar for both rates of nitrogen supply. Evidently, there was relatively more uptake of nitrogen and phosphorus in the late stages of this experiment than in the others quoted above. Some of them refer to plants grown in pots, and the restricted volume of soil in a pot may be explored so rapidly by the roots that little nutrient remains to be absorbed in the later stages of growth, whereas in the field, roots can continually penetrate

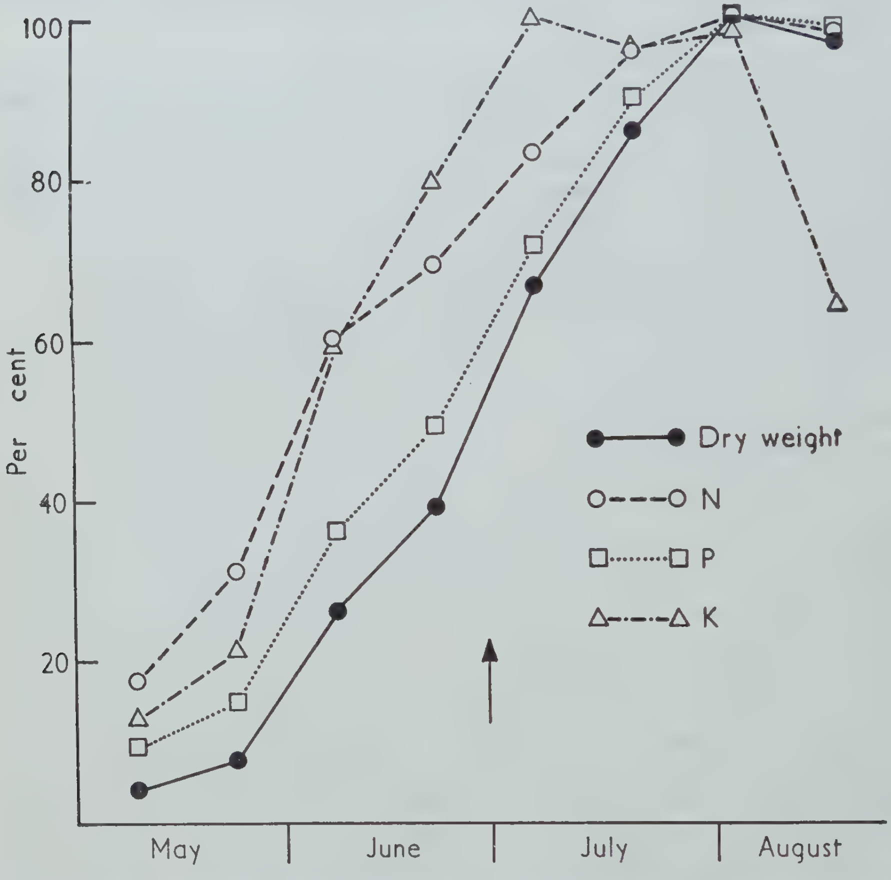

FIG. 10. The progress of nutrient absorption in the field experiment. The total quantities of nitrogen, phosphorus, and potassium present in the plants on each sampling occasion are expressed as per cent. of the maximal values; means of all varieties and nitrogen treatments.

fresh soil. Also, the progress of nutrient uptake is likely to vary on different soils; for nitrogen it probably depends greatly on the rate at which nitrogen is mineralized.

The general conclusion from this work is that the source of the higher grain yield of Proctor and Herta barleys, compared with that of Plumage-Archer, is additional photosynthesis in the ears. The evidence for this is indirect and inconclusive, depending mainly on elimination of the other possibilities. Direct evidence will be difficult to obtain, because the relative performance of the varieties in pot culture appears to differ from that in the field, so it will be necessary to devise a technique for measuring total photosynthesis by the ears of cereals that is practicable on a field scale. There is some evidence that the additional photosynthesis by the ears of Proctor and Herta was associated with a greater weight of ears at the time when they emerged and a larger surface area of ears per unit area of land, but whether it is wholly attributable to an increased amount of photosynthetic tissue in the ears, or whether increased photosynthetic efficiency of the ears, especially of Herta, is also involved, is not known.

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The Structure and Development of the Thallus in the British Species of *Gelidium* and *Pterocladia*

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With six Figures in the Text

ABSTRACT

The apical structure and the development of the thalli of all the British species of *Gelidium* and *Pterocladia* have been investigated; the development of *G. pulchellum* is described in detail. Each axis is terminated by one or more apical cells, which by their segmentation form the tissues of the thallus. An axial filament is distinct for a short distance behind each apical cell, but secondary pit-connexions develop rapidly so that in section the mature axis has the appearance of a multi-axial structure.

Lateral branches of the frond develop by the segmentation of the lateral branch apical cells, which are formed by the transformation of superficial cortical cells, either in the meristematic or mature parts of the axes. The extreme variability of external appearance is due principally to the indeterminate origin of all lateral branches.

The thallus in the British species of *Gelidium* and *Pterocladia* consists of erect fronds borne on creeping axes. The relative proportions of the frond and creeping axes in various species and their survival through adverse conditions are discussed.

INTRODUCTION

THE Gelidiales constitutes an exclusively marine order of the Florideae with a single family, the Gelidiaceae. According to Fritsch (1945), three genera are grouped in the family: *Gelidium* Lamour. s.s., *Pterocladia* J. Ag., and *Gelidiella* Feldm. Little is known of the distribution of the genus *Gelidiella*, but it would appear to be more tropical than either *Gelidium* or *Pterocladia*, species of which occur also in the temperate waters of the southern and northern hemispheres, in the latter extending northwards to the British Isles. In addition to these three genera, Kylin (1956) has referred to the Gelidiaceae a number of non-European genera of obscure affinities.

Despite the use of various species as the principal source of Agar, little is known about the family, apart from the brief account of the structure and reproduction of *Gelidium cartilagineum* (L.) Gaill. given by Kylin (1928). Numerous species have been described by various authors, but the taxonomy of the Gelidiaceae is in a chaotic condition and the identification of specimens notoriously difficult. The specific names used here are those advocated by Feldmann and Hamel (1936) in their account of the order, pending a taxonomic revision which will be published in a subsequent paper. The present paper contains an account of the development of the thallus.

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MATERIALS AND METHODS

Specimens of *Gelidium* and *Pterocladia* have been collected at frequent intervals since 1950 from various localities in the British Isles. The collections have been made principally in Anglesey, at Rhoscolyn and Rhosneigr, and the Isle of Man, at Port Erin and Port St. Mary. Other collections from Devon, Cornwall, the Channel Islands, and south-west Ireland have been made at less frequent intervals.

The material used in this investigation was fixed either in formalin-alcohol or in formalin-acetic-alcohol. No deterioration occurred in formalin-alcohol although prolonged storage tended to make the material brittle. Prolonged storage in formalin-acetic-alcohol, on the other hand, resulted in the disintegration of the tissues and it was necessary to transfer the material to 70 per cent. alcohol after 24 hours in the fixative. For microtome sectioning the required material was embedded by the standard technique in paraffin wax, using chloroform as the wax solvent. Some material required to be softened prior to embedding, in order to facilitate wax penetration and sectioning. Small pieces of the material were placed for a few minutes in a 10 per cent. solution of hydrochloric acid in 70 per cent. alcohol at 55° C. (Drew, 1945), after which they were washed in several changes of 70 per cent. alcohol. The blocks were cut at various thicknesses from 5 μ to 20 μ.

Heidenhain's iron alum haematoxylin was used principally in this anatomical investigation, with either Light Green or Orange G as the counter-stain.

THE STRUCTURE OF THE APEX IN RELATION TO THE DEVELOPMENT OF THE THALLUS

The apical structure of all the British species of *Gelidium* and *Pterocladia* has been investigated; the basic pattern of development appears to be similar in all species. *Gelidium pulchellum* (Turn.) Kütz. has been studied in greatest detail and it is on this species that the following account is based.

The erect fronds of *G. pulchellum* are from 2·0 cm. to 10·0 cm. in height (Fig. 1*a*) and arise in tufts from creeping axes. The main axis and lateral branches of the erect frond are either round or slightly flattened in section, 0·3 mm. to 1·2 mm. in diameter. A single apical cell of the type associated in this genus with lateral branches of unlimited growth is normally present in the apical meristem of each axis (Fig. 2*a*), although under certain conditions, as will be discussed later, more than one apical cell may occur. The apical cell is lenticular in shape and is easily distinguished from the surrounding cortical cells by reason of its shape, size, and method of segmentation. It is 6·0 μ to 10·0 μ in diameter and 2·5 μ to 5·0 μ in length, and it divides transversely in such a way that the apical cell and its derived segment are of approximately the same size immediately after the division. The division of the segment cell takes place soon after its formation, although in rare cases two undivided segment cells may occur below an apical cell. Each segment cell divides to produce an axial cell and four pericentral cells, of which the

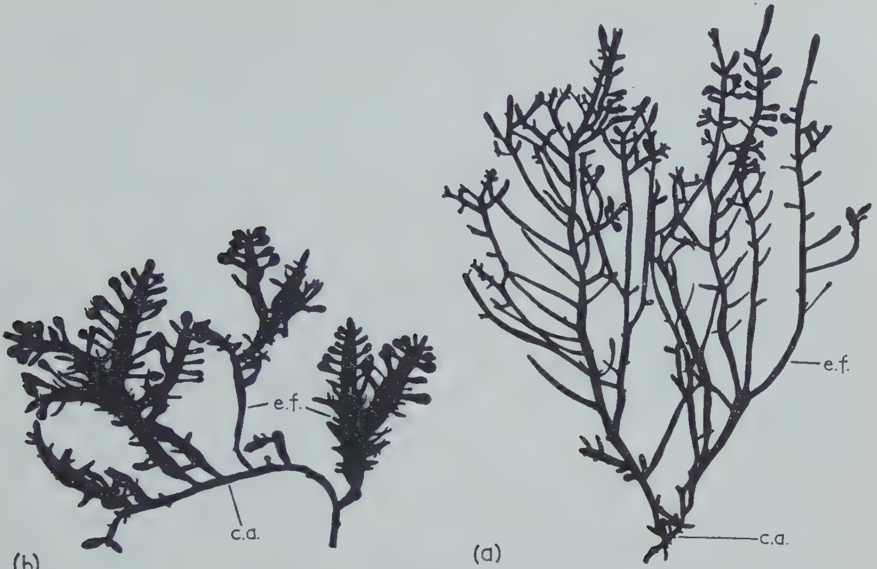

FIG. 1. The form of the thallus: (*a*) *G. pulchellum*; (*b*) *G. latifolium*; (*c.a.*, creeping axis; *e.f.*, erect frond), (× 1⅓).

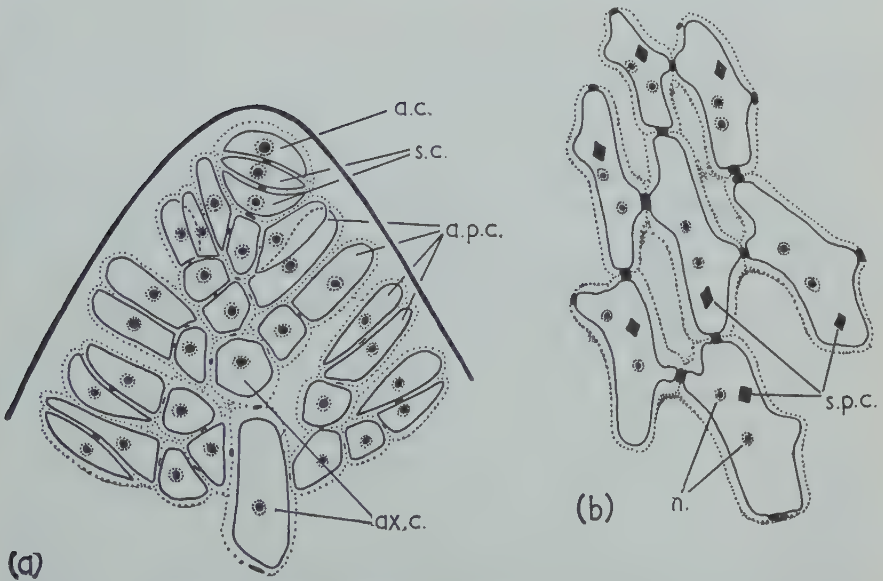

FIG. 2. *G. pulchellum*: (*a*) apical region of the thallus (*a.c.*, apical cell of the axis; *ax.c.*, axial cells; *ap.c.*, apical cells of the lateral branches of limited growth; *s.c.*, segment cell); (*b*) portion of axial plate of an older axis, showing multinucleate cells and abundant secondary pit-connexions (*n.*, nuclei; *s.p.c.*, secondary pit-connexions), (× 1,200).

pair of cells lying on the lateral flanks is formed first. The four primary pericentral cells are inclined to the axis of the thallus at an angle of 30° as a result of the concavo-convex shape of the segment cell. After elongation, the pericentral cells divide obliquely to form the apical cells of the lateral branches of limited growth, with a small quadrate cell remaining at each division. The oblique divisions of the apical cells of the lateral branches of limited growth continue, so that the axial filament is surrounded by cells, which are at first quadrate and which will constitute the medullary tissue of the axis. The divisions gradually cease, and it is the individual cells of the final order, that is, the apical cells of the lateral branches of limited growth, which constitute the outer layer of the cortex. The cortical cells are small, but contain relatively large chromatophores.

The axial cells form a filament which is distinct for only a short distance behind the apical cell, as the primary organization of the thallus is soon obscured by the formation of secondary pit-connexions. The axial cells and the other cells of the medulla are uninucleate and quadrate immediately after their formation, but rapid elongation takes place. The secondary pit-connexions develop acropetally. The nucleus of the medullary cell divides as the attenuated lobe forms at the apical pole of the cell, and one of the daughter nuclei migrates into the lobe, which is then cut off by a wall. The small cell which is thus formed fuses with the cell immediately anterior to it. During the formation of secondary pit-connexions the medullary cells continue to elongate, until in mature axes they attain a length of up to 150 μ. Secondary pit-connexions develop throughout the medullary tissue in sterile axes, but in fertile axes the development is limited. In cystocarpic lateral branches, the secondary pit-connexions develop laterally to form a plate of cells one cell in depth, whilst in tetrasporic branches the plate of cells is two to four cells in depth. These pit-connexions in the medulla are very large and of characteristic appearance. The double nature of the 'discs' can be seen in side view, whilst in surface-view the 'discs' are rhomboid in shape (Fig. 2*b*). Because of the formation of the secondary pit-connexions and their extreme elongation, the cells of the medulla are identical in cross-section and it is impossible to identify the axial filament. In consequence, the thallus has the appearance of a multiaxial structure.

Hyphae are formed at a very early stage in the development of the thallus. They arise from the basal pole of the outer medullary cells as small protuberances, which are cut off by the formation of transverse walls, so that the cells thus formed are triangular, with dense contents. Rapid elongation takes place, the initial growing basipetally between the cells of the thallus and attaining a length of up to 200 μ. The diameter of a hypha is from 2 μ to 3 μ, but the wall is thick so that the lumen is very narrow. The tip of the hypha is thin-walled and contains the nucleus which remains prominent even after the elongation of the hypha. The function of these cells is unknown, but it is generally assumed (Feldmann and Hamel, 1936) that they provide a structural support. The hyphae may occur in the medulla or in the cortex, or in both regions. As will be discussed later, various authors have attempted to use the distribution of the hyphae as a diagnostic character.

Apical growth shows a marked periodicity, which is different in the various species and which is modified to some extent by the environmental conditions. In *Gelidium pulchellum* and *G. latifolium* the division of the apical cells of the lateral branches of unlimited growth is at a maximum in the spring and early summer, whilst it is much reduced in the autumn and winter, even in well-developed specimens. Under adverse conditions the divisions occur at irregular intervals, presumably when conditions improve slightly, but the apical cells are always prominent. In *Pterocladia capillacea* the division of the apical cells of the lateral branches of unlimited growth is more restricted, with the apical cells existing for most of the year in a dormant condition. The apical cells commence to divide in the spring and growth is very rapid for a short period, during which the annual increments are added to the main axes and lateral branches of the frond. The apical cells then cease to divide and no further segmentation occurs until the following season. The newly-formed increments are at first very narrow due to rapid growth in length, while the apical cells of the lateral branches of limited growth continue to divide at a very slow rate, so that during the late summer and autumn the axes increase in length and breadth very slowly. The frond of *Pterocladia capillacea* usually attains its maximum size by December. The apical cells of the lateral branches of unlimited growth are prominent only during the period of active division.

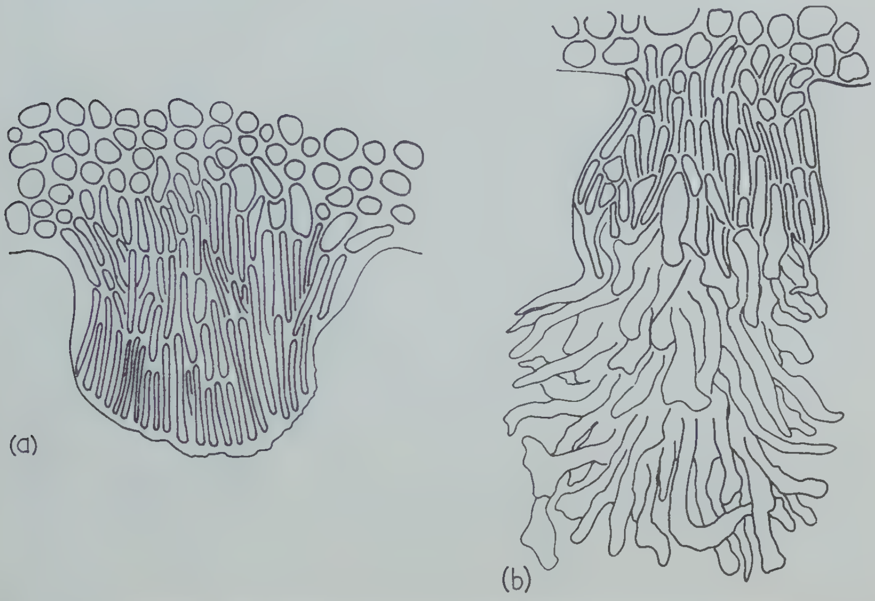

FIG. 3. *G. pulchellum*; the development of rhizoidal attachment organs from the creeping axes; (*a*) early stage of development; (*b*) fully developed rhizoidal attachment, (× 300).

The thalli of all species of *Gelidium* and *Pterocladia* have the appearance of multiaxial structures in section for the reasons already stated. In *Pterocladia* and some species of *Gelidium* (*G. sesquipedale*) the medullary cells are closely compacted so that the thallus is parenchymatous (Figs. 6 *a*, *c*), whilst in other species (*G. pulchellum*) the medullary cells are less compact and the filamentous construction of the thallus is more obvious (Figs. 5 *b–d*). As will be shown later, this difference has been used as a diagnostic character in distinguishing sterile specimens of *Gelidium* and *Pterocladia* (Okamura, 1934) but the method is not applicable in the British Isles.

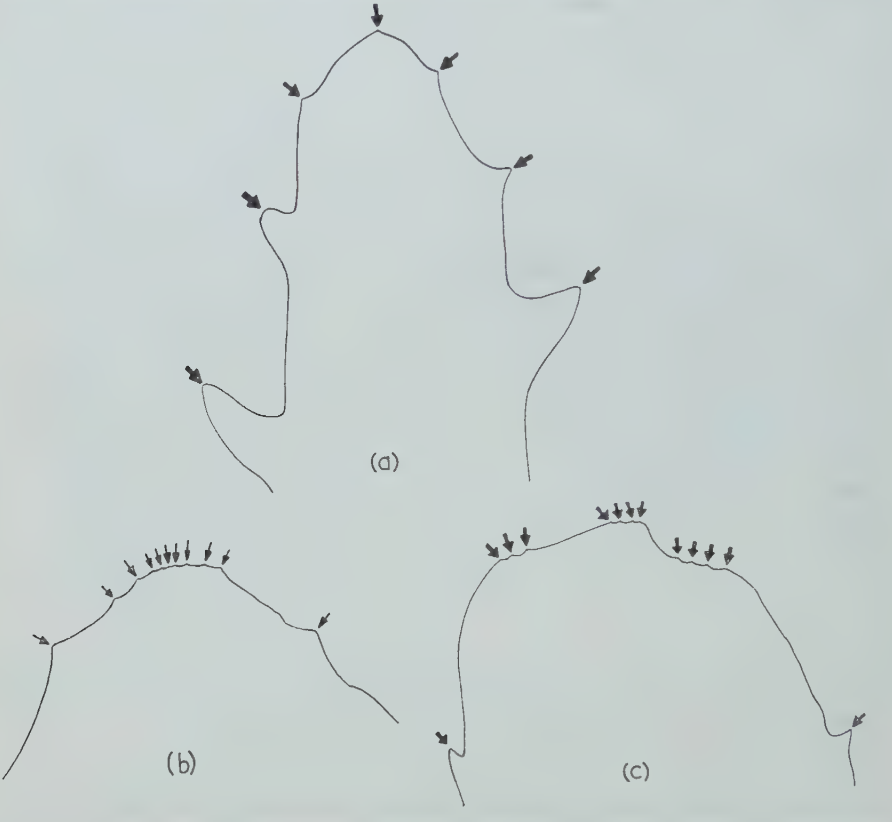

FIG. 4. The distribution of apical cells of lateral branches of unlimited growth in the apical meristem; the position of each apical cell is indicated by an arrow: (*a*) *G. pulchellum*; (*b*), (*c*) *G. latifolium*, (×25).

THE ORIGIN AND DEVELOPMENT OF LATERAL BRANCHES OF UNLIMITED GROWTH

The external appearance of the fronds of the British species of *Gelidium* and *Pterocladia* is most variable, due to the marked effect of season and environment on the development of the frond. The marked plasticity of the two genera, which has given rise to the present taxonomic difficulties, is due in part to variations in the number and position of the lateral branches of unlimited growth. A study of their origin and development is therefore desirable. The structure of the apical meristem in relation to the development of lateral branches of unlimited growth will be considered in three species: *Gelidium pulchellum, G. latifolium, Pterocladia capillacea.*

Gelidium pulchellum

The main axes of the frond of *G. pulchellum* are cylindrical and each axis is terminated by an apical meristematic region, usually with a single conspicuous apical cell at the tip. The first stage in the development of a lateral branch of unlimited growth is the initiation of its apical cell, which arises by the transformation of a cortical cell. The stages in the transformation of an apical cell of a lateral branch of limited growth, that is, a cortical cell, into an apical cell of a lateral branch of unlimited growth have not been elucidated; changes in both shape and mode of division are involved. In *G. pulchellum*, the initiation of the apical cell of a lateral branch of unlimited growth normally occurs in that portion of the apical meristem where the division of the cortical cells has almost ceased, at a distance of about 100 μ from the apical cell of the axis (Fig. 4*a*). As a result of the segmentation of the lateral branch apical cell, a small meristematic mound develops, which grows out to form a lateral branch of unlimited growth, with a structure similar to that of the main axis. Secondary pit-connexions develop in the cortex of its axis so that the medullary tissues of the main axis and the lateral branch are in continuity. As the neighbouring cortical cells have almost ceased to divide when the lateral branch apical cell is initiated, its segmentation adds little to the tissues of the main axis.

Gelidium latifolium

The axes of the fronds of *G. latifolium* are very variable in appearance; in some specimens the axes are broad and flat, whilst in others they are almost cylindrical. The apical meristem of the cylindrical axes of *G. latifolium* are similar in structure and the lateral branches arise in a similar manner to those of *G. pulchellum*. A more specialized construction occurs in the markedly flattened specimens of *G. latifolium*, where a number of apical cells of the type associated with the initiation of lateral branches of unlimited growth are dispersed over the margin of the apical meristem (Figs. 4 *b*, *c*). There are usually three to eight such apical cells present, but it is not possible to identify any one of these as the principal apical cell of the axis. The rates of division of the two types of apical cell are equal at the extremity of the meristem, but on the flanks the rate of division of the cortical cells decreases, so that the apical cell of the lateral branch and the products of its segmentation project to form the lateral branch primordium. The primordium increases in length rapidly, growing out at right-angles to the axis to form a lateral branch.

The lateral branch primordium contains a single apical cell at first, and if this condition persists the development of the branch will be restricted. The branch will be cylindrical or slightly compressed and any secondary lateral branches which may develop upon it will be microscopic in size. In certain cases, usually in the spring when growth is rapid, the apical meristem of the lateral branch increases in breadth and further apical cells develop so that it resembles the apical meristem of the main axis. In such a case, the developing primary lateral branch is as broad as the main axis and it repeats the structure of the main axis, with the apical cells developing into branches of a second order. A primary lateral branch of this type frequently shows a marked constriction at the point of insertion on the main axis, due to its origin from a slender primordium.

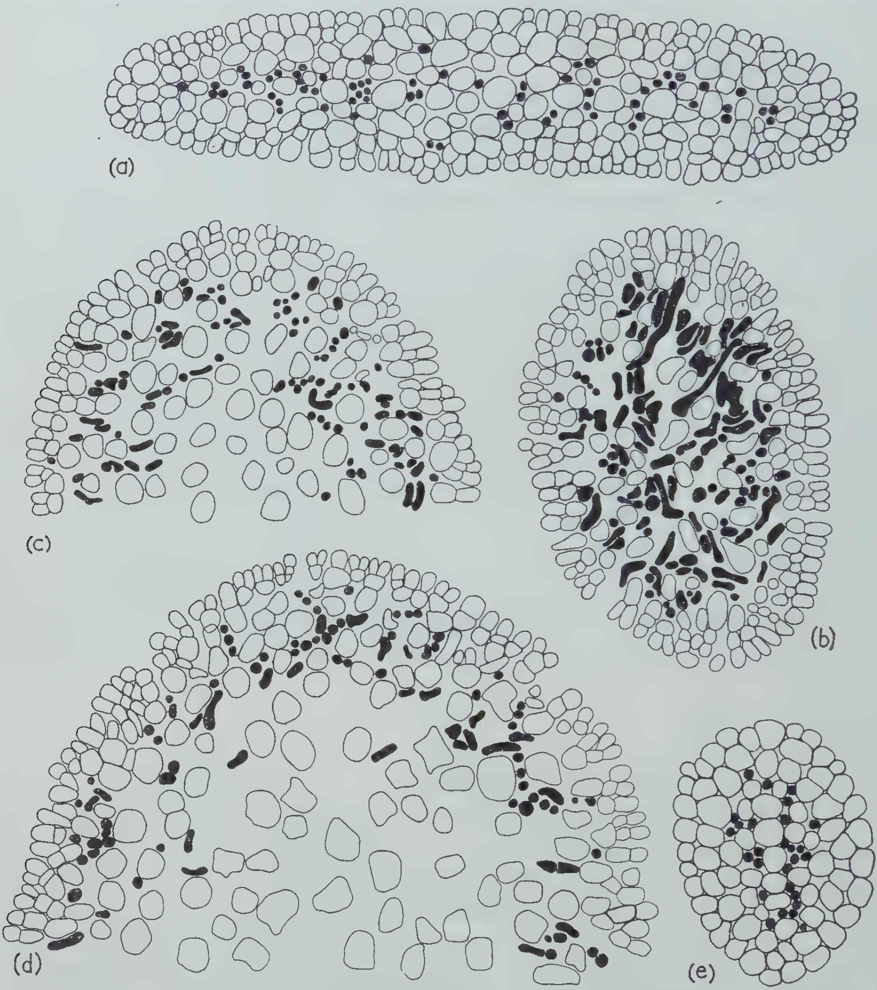

FIG. 5. *G. pulchellum*; the distribution of hyphae, the hyphae in silhouette, other cells in outline: (*b*), (*c*), (*d*) sections through progressively older parts of the same axis; (*e*) section through creeping axis; (*a*) section through the flattened tip of an axis, (× 45).

When growth is rapid, the apical meristematic region of a main axis of

G. latifolium, with its many apical cells, may become divided into two or more segments, each of which resembles the main axis in the structure of its apical meristem and the manner in which the lateral branches of further orders are borne. These segments form lateral branches which differ from those described previously, in that they do not exhibit a constriction at their point of insertion on the original axis, as they have not developed from a slender primordium, initiated by a single apical cell. In those axes of *G. latifolium* in which the apical meristem contains a number of apical cells of lateral branches of unlimited growth, these apical cells contribute to the tissues of the main axis before growing out to form lateral branches.

There is no fundamental distinction between the two types of apical construction, as the differences can be correlated with the relative rates of development of the main axes, lateral branches of limited growth, and lateral branches of unlimited growth, which in turn can be correlated with environmental conditions. Under adverse conditions, the lateral branch primordia are formed at considerable time intervals, so that they are initiated at some distance from the principal apical cell of the main axis, which is thus always distinct. Under more favourable conditions, the lateral branch primordia arise in profusion in the apical meristematic region in close proximity to the apical cell, obscuring it by their development. That there is no fundamental distinction between the two types of construction is supported by the evidence that intermediate stages occur frequently and that the structure of the apical meristematic region of any frond may alternate from the one type of construction to the other during the year.

Pterocladia capillacea

The mature axes of *Pterocladia capillacea* are as broad as the axes of the most luxuriant specimens of *G. latifolium* and although the basic pattern of development is the same in the two species, there are considerable differences in detail. In *Pterocladia* there is a single apical cell present in each main axis, and apical cells of the lateral branches of unlimited growth are always initiated some distance behind the apical cells of the main axes, as in *G. pulchellum.* The apical cells of the main axes commence to divide in the spring and for a short period very rapid elongation takes place, reaching a maximum of 5 to 8 mm. per week. The increments added to the main axes and the lateral branches of the frond are cylindrical when first formed, 600 to 900 μ in diameter. The axes then increase in length and breadth, reaching a maximum size (*c.* 3 mm.) by December, as a result of the continued division of the cortical cells, that is, the apical cells of the lateral branches of limited growth. The apical cells of the main axes become overgrown by the excessive cortical segmentation and are very difficult to detect during the winter. It could not be determined whether the same apical cells persist in the principal axes for several reasons, or whether new apical cells are initiated each year. The irregular disposition of the new increments in the apical regions of the axes supports the latter view. The basic pattern of the development of the frond is thus the same as in *Gelidium*, but there are considerable differences in detail.

Adventitious lateral branches of unlimited growth. In the Gelidiaceae, lateral branches of unlimited growth normally develop in relation to the apical meristem of the axis on which they are borne, although adventitious lateral branches develop frequently on mature axes. The development of adventitious lateral branches of unlimited growth appears to be the result either of abnormal environmental conditions or of regeneration from an injured frond. In certain specimens of *G. pulchellum* growing in shallow pools on Holy Island, Anglesey, the fronds appeared normal during the winter, but the axes became clothed with adventitious branches of varying length in the spring, when the pools dried up at each low tide. Similarly in fronds which survive through the winter, adventitious lateral branches may be formed by regeneration from the main axis or from the stump of an old lateral branch. Regeneration occurs frequently in *Pt. capillacea* and the larger specimens of *G. latifolium*, and also in many of the smaller species as a result of animal grazing. Adventitious lateral branches develop in a similar manner to normal branches of unlimited growth, by the outgrowth of an apical cell formed by the modification of a cortical cell.

The indeterminate origin of all lateral branches of unlimited growth in the three species considered is the most significant conclusion which can be drawn from the present investigation. Undoubtedly, the variation in the number and disposition of the lateral branches of unlimited growth is the principal cause of the characteristic variable external appearance of the fronds of the species of *Gelidium* and *Pterocladia*.

THE CREEPING AXES AND THEIR RELATION TO THE ERECT FROND

The thalli of species of *Gelidium* and *Pterocladia* consist of creeping axes on which the erect fronds are borne at intervals (Figs. 1 *a*, *b*). The creeping axes of *Gelidium* are from 150 μ to 600 μ in diameter and deep crimson in colour. The meristematic tip of each creeping axis contains a single apical cell. The structure of the creeping axes and of their apical meristems and the segmentation of their apical cells are all similar to those of the erect fronds. The creeping axes are attached to the substrate by outgrowths from the ventral side of the cortex (Fig. 3) and the erect fronds arise as lateral branches in the same way that lateral branches of unlimited growth are formed in the erect frond. The creeping axes of *Pt. capillacea* are thicker, less deeply coloured, and relatively poorly developed. In this species the erect fronds arise at short intervals, so that the plants often appear to be tufted. The origin of the erect fronds from the creeping axes has not been followed.

The relative development of the erect fronds and creeping axes varies not only from species to species, but also in certain cases in the same species. For instance, specimens of *G. latifolium* occur at Galway, between Gratton Road and Mutton Island, either on long reefs of rock or attached to small stones embedded in stretches of sandy mud between the reefs. On the reefs the erect fronds arise at intervals from well-developed creeping axes, whereas the specimens attached to the stones are tufted, with minute creeping axes.

Careful examination shows that fronds which can be assigned to different 'species' often develop from the same creeping axis. The taxonomic significance of this observation will be discussed in later papers.

THE PERENNATION OF THE ERECT FRONDS AND THE CREEPING AXES

The perennation of the cystocarpic and tetrasporic phases of *Gelidium* is a fact which has been neglected completely by previous authors. In view of the discussions concerning the relative frequency of occurrence of the cystocarpic and tetrasporic phases of some species (Feldmann and Hamel, 1936), it would appear that data on the life-span of the phases would be relevant.

The erect frond may persist for four or five years in some species, e.g. *Pt. capillacea*, *G. sesquipedale*. Each year, increments (including lateral branches) are added to the main axes in these two species, and although the lower lateral branches may persist, they usually fall away so that the specimens are frequently dendroid in appearance. The two species which behave in this manner occur either sub-littorally or above low-water mark in deep pools. Most species which occur in exposed situations produce new fronds annually in the spring. The amount of growth of the frond of *G. latifolium* and the time of its persistence are dependent upon the environmental conditions. The erect fronds seldom persist for more than one year in Anglesey and the Isle of Man, although two-year fronds may be found occasionally. At Ardfry, at the head of Galway Bay, where the shore is terraced, the specimens from the upper terraces are annual, whilst those from the lower terraces, which are never exposed even during spring tides, are larger, and persist for about three years.

The creeping axes are remarkably persistent in all species. Some reduced forms, previously considered as *G. pusillum*, consist of little more than the creeping axes. The plant is capable of existing in this condition for a considerable time under unfavourable conditions, but when conditions improve, erect fronds are formed.

A pool at Borth Saint (Holy Island, Anglesey), shaded during the winter months, when it contains a few cm. of water, but no longer shaded during the summer, so that frequently it dried up between successive high tides, has been watched between 1950 and 1955. Each autumn, small erect fronds of the winter state of *G. pulchellum* have developed and survived until April. The sides of the pool are bare during the summer months, but examination showed that the creeping axes were surviving in crevices in the rock, beneath a covering of *Lithothamnion*.

In *G. latifolium* also, the production of erect fronds over a period of years from the same creeping axes has been demonstrated. Sexual plants of this species are relatively rare in Great Britain, but at Borth Saint a careful search for them was undertaken between 1950 and 1955. During this time cystocarpic plants were found on only one small area of rock. In 1950 all the erect fronds were removed, leaving only the creeping axes. The area was marked and in the three succeeding years cystocarpic fronds developed and were removed. No diminution in the vigour of the plants could be detected despite this extensive cropping. When the area was visited in 1955 it was completely bare, but, in the interval, the environmental conditions had changed considerably due to a breach in the protecting reef.

DISCUSSION

Floridean thalli of all degrees of complexity are built up of aggregations of branch systems in which main axes of unlimited growth and lateral branches of limited growth can be distinguished. In the uniaxial genera, the lateral branches of limited growth may be loosely aggregated, as in *Batrachospermum*, or compact as in *Plocamium* and *Gelidium*. In *Gelidium* the single axial filament is distinct for only a short distance behind the apical cell, and as a result of the compact nature of the thallus, the marked elongation of the cells of the medulla, and the abundant formation of secondary pit-connexions, the adult portions of the thallus are parenchymatous in structure.

The lateral branches of unlimited growth in the uniaxial genera of the Florideae may develop either by the continued growth of a lateral branch of limited growth, as in *Lemanea* (Dixon, unpublished), or arise *de novo* as in *Gelidium*. The indeterminate origin of the lateral branches of unlimited growth in *Gelidium*, together with the possible persistence of the erect frond for several years, and the regeneration of further lateral branches of unlimited growth from old axes, lead to the diversity of appearance characteristic of this genus. The interpretation of this plastic material by algologists who have regarded any variation from the type specimen of a given species as a new form variety, or even species, has resulted in taxonomic chaos. Application of the data presented here will facilitate the elucidation of many of these difficulties.

A single apical cell terminates each of the branches of unlimited growth in *Gelidium*, but in the flattened thalli of *G. latifolium* a more specialized construction may occur, in which from four to twelve apical cells are disposed over the margin of the apical region. The apical regions of these flattened thalli resemble those of certain genera of the Delesseriaceae and Gigartinales, to which the term 'marginal meristem' has been applied. Little is known of these 'marginal meristems', but it would appear that there are two distinct types of structure described under that name. The 'marginal meristem' of a mature axis of *Nitophyllum punctatum* (Kylin, 1923) is made up exclusively of cortical cells, that is, apical cells of lateral branches of limited growth. This is probably a peculiar development associated with the extensive intercalary cell-division which occurs in *Nitophyllum* and related genera, and which bears no relation to the development of lateral branches of unlimited growth. In *Rhodophyllis bifida*, on the other hand, the 'marginal meristem' includes, in addition to the cortical cells, a number of apical cells of the type associated with lateral branches of unlimited growth, which grow out eventually to form lateral branches of the thallus. The apical structure of *R. bifida* is thus similar to that of *G. latifolium*. It is clear that the peculiar meristematic condition of these two latter species is the result of the increased inception of apical cells of lateral branches of unlimited growth during a period of rapid development, and represents nothing more than a transitory condensation of the normal type of construction. A fuller investigation of all 'marginal meristems' is obviously necessary, but in view of the transitory nature of the 'marginal meristems' of *Gelidium* and *Rhodophyllis*, the term should not be applied to these structures, but should be restricted for the meristematic construction of the Delesseriaceae.

The early stages of the germination of the carpospores and tetraspores of *Gelidium* have been described by Killian (1914), but attempts to germinate the spores of the various British species have not been successful. Ueda and Katada (1949) have described the early development of young plants of the Japanese species *Gelidium amansii* and have shown that the lateral branches which develop from the basal part of the main axis may either grow erect, or grow horizontally to form creeping axes on which further erect fronds will develop later. These observations probably explain the variation in the development of the creeping axes described in the specimens from Galway.

No germlings have been detected in the localities investigated in the British Isles and all young plants could be traced back to creeping axes. Cystocarpic fronds are very rare in all the British species of *Gelidium*, whilst in *Pterocladia* both tetrasporangia and cystocarps are unknown in the British Isles. It would appear likely from this data that many of the species involved do not exhibit a regular alternation of somatic phases, or if such an alternation does occur, it is obscured by the extensive production of erect fronds from persistent creeping axes.

Many of the original descriptions of species of *Gelidium* and *Pterocladia* were based on sterile specimens so that the later discovery of reproductive structures frequently resulted in major taxonomic rearrangements. For instance, the discovery of unilocular cystocarps in *Gelidium capillaceum* resulted in the transference of that species to the genus *Pterocladia*, created by Agardh (1851) for the Australasian species *Pt. lucida*, on account of its unilocular cystocarps. The difference in the structure of the cystocarp, which is bilocular in *Gelidium* and unilocular in *Pterocladia*, has been used in dichotomous keys by various authors (e.g. Newton, 1931) to distinguish between the two genera. In all parts of the world the method is only of very limited application, because of the relatively large numbers of sterile specimens, but in the British Isles it is virtually useless, due to the extreme rarity of cystocarpic plants of *Gelidium* and the apparent absence of cystocarpic plants of *Pterocladia*. Attempts have been made by various authors to correlate other anatomical features with certain species or groups of species, in order to identify the numerous sterile specimens of the two genera. The validity of many of the proposals is doubtful, due to the lack of an overall anatomical investigation of the Gelidiaceae, so that, before a revision of the family could be undertaken, a critical survey of the taxonomic significance of these features is necessary. The two features which have received most attention are, firstly, the structure of the medulla, and secondly, the distribution of hyphae.

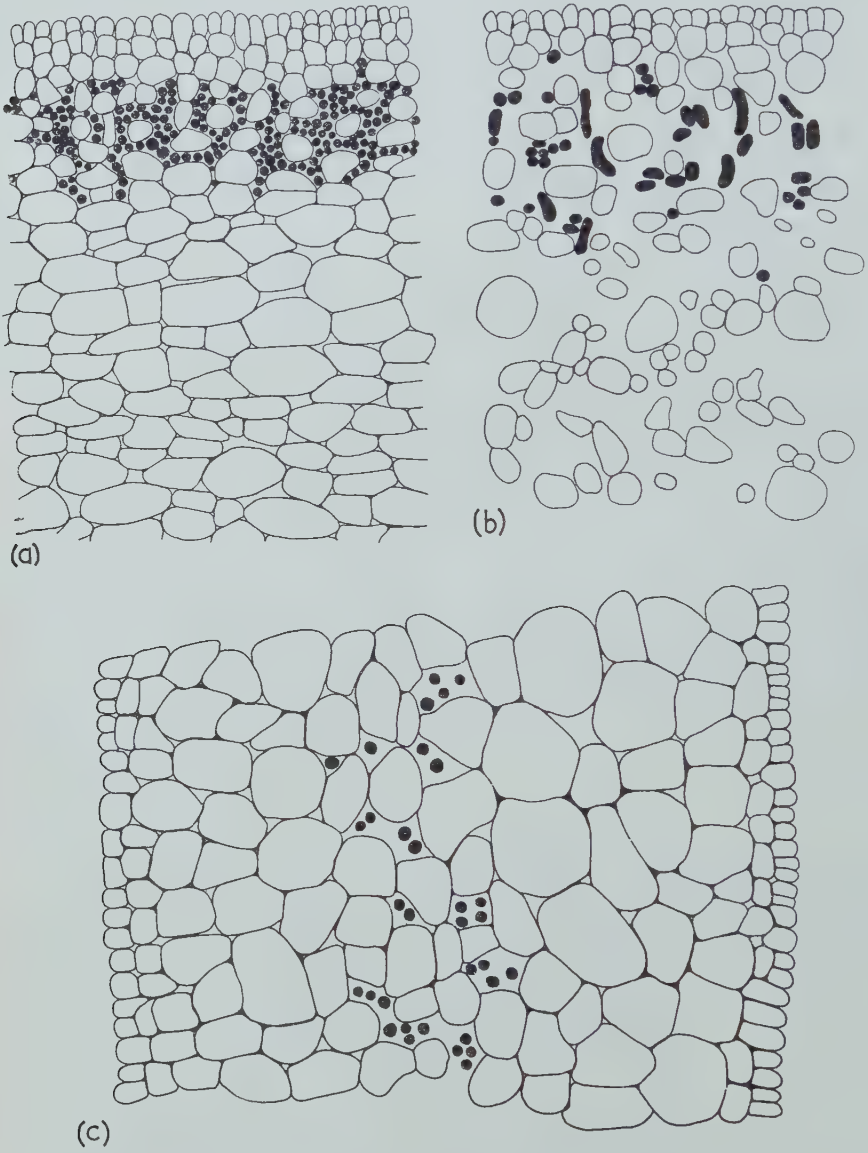

FIG. 6. Distribution of hyphae; the hyphae in silhouette, other cells in outline; (*a*) *G. sesquipedale*; (*b*) *G. latifolium*; (*c*) *Pt. capillacea*, (×45).

Okamura (1934) claimed that the Japanese specimens of *Gelidium* and *Pterocladia* could always be distinguished by the structure of the medulla.

He claimed that in *Pterocladia* the medulla is compact and parenchymatous, whilst in *Gelidium* it is clearly filamentous in structure. Data on this feature from other regions are not available, but such a correlation does not exist in the British Isles. A compact medulla is found in *G. sesquipedale* (Fig. 6*a*), which is similar to that of *Pt. capillacea* (Fig. 6*c*), whilst in some other species of *Gelidium* the structure of the medulla changes during the life of the frond. In *G. latifolium* a filamentous medulla occurs in first-year fronds, whilst in older fronds the medulla is compact and parenchymatous. The structure of the medulla is therefore of little assistance in distinguishing between sterile specimens of *Gelidium* and *Pterocladia* in the British Isles.

Gardner (1927), in a survey of some species of *Gelidium* of the Pacific coast of North America, indicated the distribution of the hyphae for each species, showing that these structures were distributed either in the medulla, or in the outer cortex, or in both regions of the axes. More recently, Okamura (1934) applied this system for the identification of sterile specimens, claiming that in the Japanese specimens the hyphae are grouped in the outer part of the medulla in all species of *Gelidium* (other than *G. pusillum*), whereas in *G. pusillum* and the species of *Pterocladia* the hyphae occur in the centre of the medulla. Feldmann and Hamel (1936) also follow the classification of Gardner and divide the French species into three groups according to the distribution of the hyphae, but admit that considerable variation can occur. During this investigation it has been found that the distribution of the hyphae in most species is exceedingly variable, although in some species it is very characteristic. For instance, in *G. pulchellum* the hyphae occur in the outer medulla in well-developed specimens (Fig. 5*d*), or extend into the innermost parts in younger specimens (Fig. 5*c*). In *Pt. capillacea*, on the other hand, the hyphae occur exclusively in the inner medulla (Fig. 6*c*). The latter characteristic distribution is very useful for the identification of sterile specimens of this species, as the hyphae occur in the outer medulla in those large species of *Gelidium* with which it is likely to be confused. The method is unfortunately of very little use for the separation of the smaller species of *Gelidium*, due to the extreme variability of the distribution of the hyphae. Apart from the identification of *Pterocladia* in this way, the use of internal anatomical features for the identification of sterile specimens is of little assistance.

Further investigations of the cytology, taxonomy, and life-history of the British species of *Gelidium* and *Pterocladia* are now in progress.

ACKNOWLEDGEMENTS

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Drought-resistance in *Pelargonium zonale*

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With two Plates and three Figures in the Text

ABSTRACT

The small persistent leaves produced by *Pelargonium* when water is withheld have been investigated in respect of their production, structure, carbohydrate changes, and transpiration characteristics. It is concluded that these leaves show no special adaptations, but are merely normal leaves arrested in an immature condition. Their survival during drought is attributed to the incomplete vacuolation of the cells, and the findings are consistent with Iljin's views on the importance of mechanical protoplasmic damage, caused by excessive shrinking, in drought-injury.

1. INTRODUCTION

THE literature on drought-resistance has been critically reviewed both by Levitt (1956) and by Iljin (1957), and no general references will be given here. In view of the many comprehensive investigations of this type already available some apology is needed for yet another; and our starting-point will be Runyon's (1936) statement that 'Development [of the leaves of *Larrea*] during a time of water-shortage seems to engender drought enduring capacity, while attainment of maximum size and water-content entails loss of this capacity.' The small, tough, persistent dark-green leaves produced by *Pelargonium* when water is withheld have already been the subject of a communication on leaf-area growth (Amer and Williams, 1957), and we believed that a comparison between these and normal leaves (whose water-relations are reasonably well understood—Williams and Amer, 1957) might throw some light on the nature of this drought-enduring capacity. To this end we have used as many techniques, morphological and physiological, as are available to us; and we have had the privilege of making certain necessary observations on the electron microscope in the Sub-Department of Plant Biophysics of the University of Leeds. In this paper we present only our main findings; a fuller account of the work (excluding the electron microscope observations) will be found in the original thesis (Amer, 1955).

2. GROWTH AND LEAF PRODUCTION

Potted plants of *Pelargonium zonale* var. 'Paul Crampel' were grown under a variety of watering régimes, the most important being: (1) normal daily watering; (2) normal watering until cuttings were satisfactorily established

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(carrying 8 to 10 leaves) then no further watering; (3) as (2), but after 5 weeks' drying, normal daily watering resumed. The general appearance of 'wet' and 'dry' plants is shown in Plate 1, in which the characteristic tuft of drought-enduring leaves is clearly shown in the unwatered plants.

Leaf-production and leaf-fall are summarized in Table 1, and the following points should be noted: (1) Mature leaves, which show a plastochron of about 1 week and normally persist for about 16 weeks, are quickly shed on drying; (2) the 'dry' leaves represent those leaves of the main sequence produced 2 or 3 weeks either side of the onset of drying; (3) irrigation after a period of drying results in a rate of leaf production *higher* than normal (cf. Arney, 1953, for *Fragaria*). The second of these points suggests strongly that the 'dry' leaves are essentially normal in early development, but suffer some check and/or modification at the onset of what would normally be the extension period. On reirrigation they expand rapidly, though not quite to normal size (*vide* the values of K in Amer and Williams, 1957, Table 1); in this respect they may be said to be less than normally extensible, which supports the observations of Clark and Levitt (1956) for *Soja*.

TABLE 1

Leaf-production and Leaf-fall under Different Watering Régimes

(All figures represent numbers of leaves)

	Wet			Dry			5 weeks wet, then dry			5 weeks dry, then wet		
Week	New	Fallen	Total	New	Fallen	Total	New	Fallen	Total	New	Fallen	Total
0	—	—	8	—	—	8	—	—	7	—	—	6
1	1	—	9	1	—	9	2	—	9	2	—	8
2	1	—	10	1	2	8	1	—	10	—	1	7
3	1	—	11	—	1	7	1	—	11	—	1	6
4	1	—	12	—	1	6	1	1	11	—	2	4
5	2	—	14	—	—	6	1	—	12	—	—	4
6	1	—	15	—	—	6	—	3	9	1	—	5
7	1	—	16	—	—	6	1	1	9	1	—	6
8	1	1	16	—	1	5	—	1	8	2	—	8
9	1	—	17	—	—	5	—	—	8	1	—	9
10	1	—	18	—	—	5	—	1	7	1	—	10
11	1	—	19	—	—	5	—	1	6	2	—	12
12	1	—	20	—	—	5	—	1	5	1	—	13
13	—	—	20	—	—	5	—	—	5	1	—	14
14	2	—	22	—	—	5	—	—	5	2	—	16
15	1	1	22	—	—	5	—	—	5	1	—	17
16	1	1	22	—	—	5	—	—	5	1	—	18

Weekly increments of stem-length under the three standard treatments are shown in Fig. 1, and again we note the abnormally high rate of growth on reirrigation after drying, which enables the temporarily droughted plants to 'catch up' with those continuously irrigated. This property has been reported so frequently for such a wide variety of species that it may be regarded as an inherent property of drought-resistant plants; so far as we are aware it has never been satisfactorily explained.

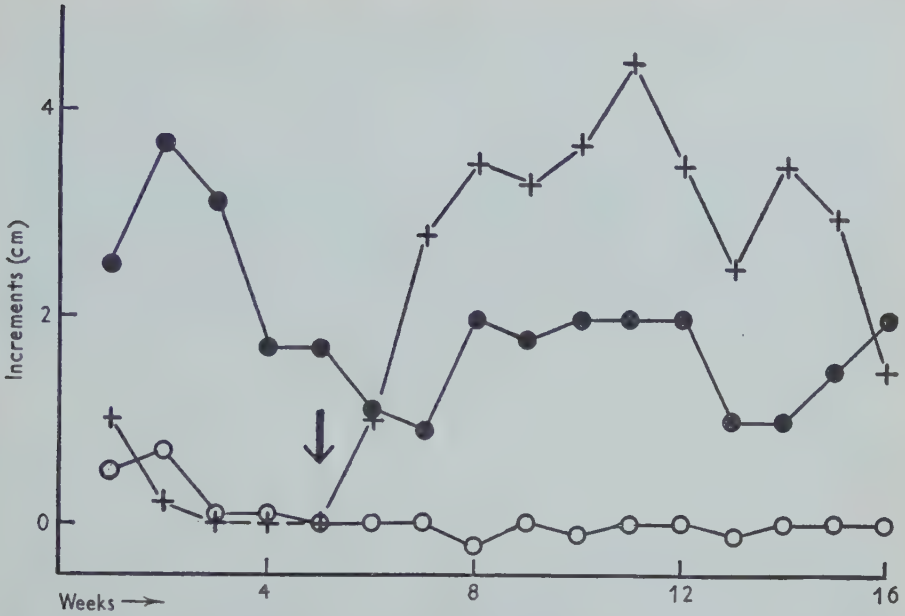

FIG. 1. Effect of watering régime on weekly increments of stem-growth. Closed circles, normally watered ('wet') plants; open circles, 'dry' plants; crosses, plants dry for 5 weeks then watered (at arrow).

3. LEAF ANATOMY

Quantitative anatomical information in respect of typical leaves under the three standard treatments is given in Table 2, which includes also data for an immature 'wet' leaf of about the same diameter (2·5 cm.) as that of the 'dry' leaf. Cell counts were obtained by maceration and haemacytometer counts (Brown and Rickless, 1949); guard-cell starch was estimated by the scoring method of Williams and Barrett (1953); other measurements were obtained by conventional microscopical techniques.

TABLE 2

Anatomical Data

(Epidermal and stomatal data refer to lower epidermis only)

	'Dry'	Immature 'wet'	Mature 'wet'	'Dry'—irrigated
Leaf area, cm.^{2}	30·8	36·9	65·5	52·0
No. of cells per cm.^{2} disc	$4{\cdot}9 \times 10^{6}$	$3{\cdot}7 \times 10^{6}$	$2{\cdot}6 \times 10^{6}$	$2{\cdot}7 \times 10^{6}$
Total no. of cells per leaf	$1{\cdot}5 \times 10^{8}$	$1{\cdot}4 \times 10^{8}$	$1{\cdot}7 \times 10^{8}$	$1{\cdot}4 \times 10^{8}$
Area of epidermal cell, μ^2 (mean of 20)	476	586	1,121	769
Stomata per cm.^{2}	$4{\cdot}66 \times 10^{4}$	$2{\cdot}52 \times 10^{4}$	$2{\cdot}10 \times 10^{4}$	$2{\cdot}20 \times 10^{4}$
Stomata per leaf	$1{\cdot}43 \times 10^{6}$	$0{\cdot}93 \times 10^{6}$	$1{\cdot}37 \times 10^{6}$	$1{\cdot}14 \times 10^{6}$
Stomatal dimensions, μ (mean of 40)	24×17	27×22	30×26	25×20
Stomatal starch area μ^2 (mean of 40)	292·6	205·9	38·4	188·1
Pore width μ (mean of 40)	0	2·8	6·2	3·1

The 'dry' leaves show the usual characteristics: small epidermal cells, and high numbers of cells and stomata per unit area; but when these are reduced to a 'per leaf' basis, it will be seen that there is very little difference. The dimensions of individual stomata also show little difference—it is clear that even under fully irrigated conditions they extend very little in comparison with the thin-walled epidermal cells. The remaining stomatal observations—closure under dry conditions, high starch-content in immature and 'dry' leaves—are of a type long familiar, whose significance has been extensively discussed in stomatal literature. Now, although the often-quoted statement that increase in leaf area after emergence from a bud is due only to extension is probably an exaggeration, the table suggests that in these leaves, at least, cell-division is substantially completed; and from this point of view the 'dry' leaves might simply be normal leaves which have failed to extend.

However, Fig. 2 shows that there is at least one larger-scale anatomical feature that still requires explanation: the increased palisade development under dry conditions. We believe that this familiar observation may be explained in part at least by mechanical considerations arising out of the detailed studies on leaf development by Noak (1922) for *Pelargonium* and Avery (1933) for tobacco. The sequence of cessation of cell-division is, roughly, epidermis first, then middle and lower mesophyll, then palisade; extension stops first in the spongy mesophyll, whose cells are mechanically separated by the still-extending epidermis, which itself ceases to extend at a time when the palisade cells may still be capable of further division. Now, under dry conditions the epidermis may be expected to be the first to suffer, partly because it is on the outside, and partly because it probably receives its water-supply direct from the main veins (*vide* La Rue 1930, Wylie 1943, Armacost 1944); and lateral growth of the leaf must cease with that of the epidermis. Any further division and extension can only be *vertical*, and might well result in some of the lower mesophyll being disposed in a palisade-like manner. This is supported by a comparison of Figs. 2 (*a*) and (*c*), from which it will be seen that the 3—in some places 4—palisade layers of the mature 'dry' leaf are reduced to 2 on irrigation.

4. BIOCHEMISTRY

It seems clear that much of the very extensive previous work on the carbohydrate-, nitrogen-, and pigment-fractions of droughted plants has been undertaken in the hope that it would reveal some general change in biochemical make-up which would provide an explanation of the ability to endure drought, or at least of the ability to grow at an abnormally high rate when drought is ultimately relieved. The diversity of results obtained by different investigators with different plants (*vide* thesis, Amer, 1955, for detailed citations) shows that this hope is vain. Nevertheless, such data do at least provide information as to the metabolic status of the organs concerned; and, lacking the equipment that would be required to obtain direct measure-

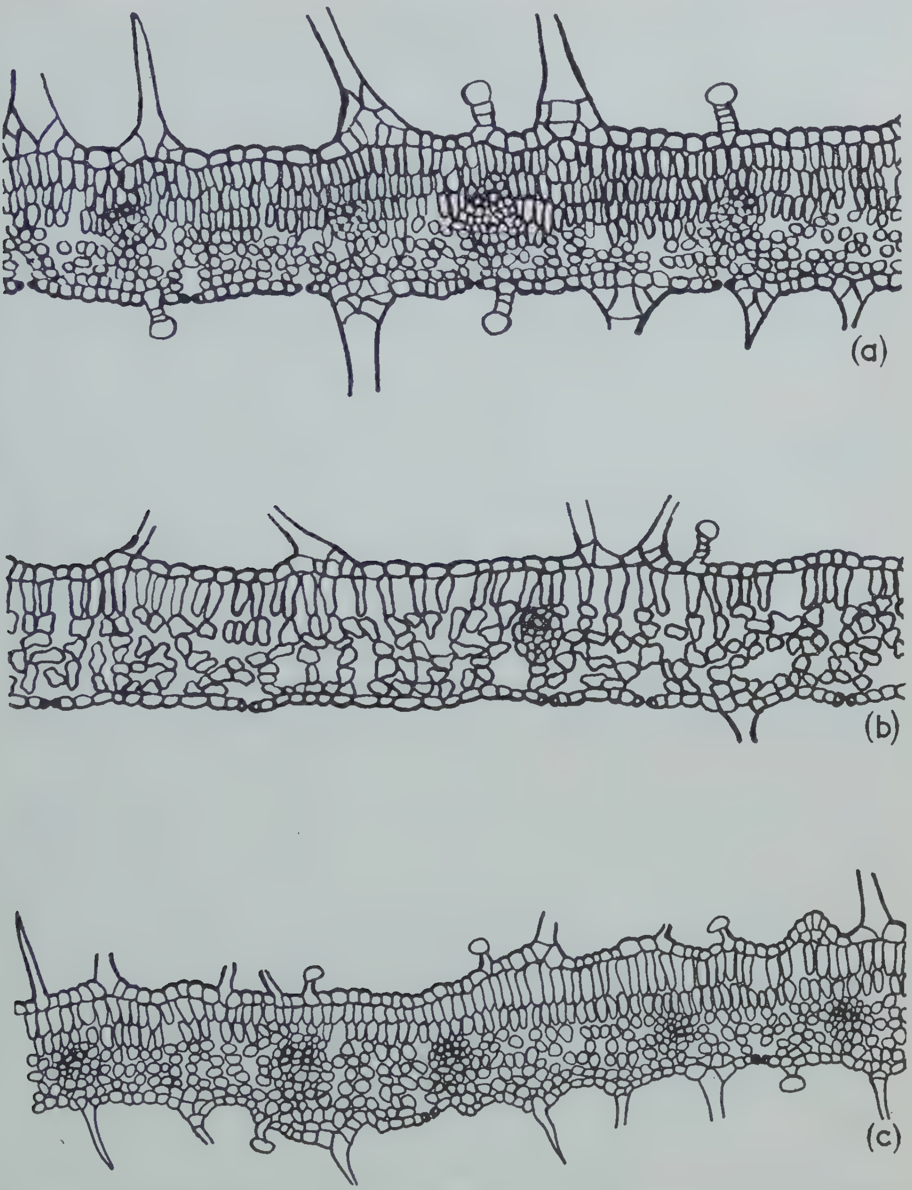

FIG. 2. Transverse sections of leaves under different watering régimes, all to the same scale; (*a*) 'dry'; (*b*) 'wet'; (*c*) dry, then watered. (All *c.* ×90.)

ments of gas-exchange in 'dry' leaves, we have examined the pigment and carbohydrate fractions with this end in view.

The methods are of no intrinsic interest, and are given in the thesis; those selected for sucrose and starch proved somewhat unsatisfactory, and the results we present for these in Table 3 are probably slight underestimates.

TABLE 3

Biochemical Data

(Fresh weight in g./leaf; all other constituents in mg./leaf. Values obtained during the 'dry' régime are italicized)

Weeks		2	4	6	8	12	16	20	24	28	32
Residue	wet	82·0	129·5	134·0	179·0	—	—	—	—	—	—
	dry-wet	—	*100·0*	—	*116·8*	*104·8*	*86·0*	121·0	136·5	117·0	114·5
Starch	wet	13·4	16·4	22·4	50·8	—	—	—	—	—	—
	dry-wet	—	*7·4*	—	*9·2*	*7·7*	*6·7*	21·8	15·0	20·6	5·8
Sucrose	wet	3·7	2·6	6·0	5·9	—	—	—	—	—	—
	dry-wet	—	*2·5*	—	*1·9*	*1·4*	*1·3*	5·5	6·6	3·3	2·2
Reducing sugar	wet	9·9	8·9	11·3	12·6	—	—	—	—	—	—
	dry-wet	—	*7·5*	—	*7·6*	*4·8*	*3·6*	11·1	12·7	11·5	10·6
Chlorophyll	wet	5·2	9·2	10·4	11·5	—	—	—	—	—	—
	dry-wet	—	*7·2*	—	*8·2*	*8·0*	*8·3*	8·4	8·9	6·6	8·5
'Carotene'	wet	1·0	1·7	2·2	2·6	—	—	—	—	—	—
	dry-wet	—	*1·7*	—	*2·3*	*1·6*	*2·0*	2·1	1·6	1·6	2·8
'Xanthophyll'	wet	2·6	4·3	4·1	3·4	—	—	—	—	—	—
	dry-wet	—	*2·9*	—	*3·7*	*3·8*	*3·6*	2·0	4·4	5·0	5·1
Fresh weight	wet	0·89	1·97	2·51	3·47	—	—	—	—	—	—
	dry-wet	—	*1·28*	—	*1·62*	*1·11*	*1·01*	2·10	2·56	1·55	1·85

Nevertheless, errors of this type are small compared with those between the (2 or 4) replicates, which always represent separate leaves. The 'wet' values, which give an indication of normal maturation, represent the 2nd, 4th, 6th, and 8th leaves from the apex on a single shoot, and are inserted in the table on the assumption that the plastochron is exactly 1 week. The 'dry' values (italicized) represent sample leaves taken at 4-week intervals from a *group* of plants from which water was withheld at week 0; after 16 weeks these plants were regularly watered and 4-week samples taken for a further 16 weeks.

Pigment changes are small, and may be neglected; evidently the full pigment complement is formed early in leaf development and changes little thereafter, the darker appearance being due to the greater concentration of pigment. (We have made no attempt to extract anthocyanins; these commonly increase under dry conditions (Blank, 1947), and may contribute to the colour.) Nor is there much suggestion that the pigments are drawn upon during the 'dry' period, though other observations (not presented here) suggest that if the drying is prolonged for a further 10 weeks, the pigment levels fall.

The carbohydrates are held during the 'dry' period at a level roughly corresponding to that of a normal leaf at an early stage of development. Starch remains remarkably constant; 'residue' does not merit detailed discussion, since it is necessarily contaminated to an unknown extent by insoluble nitrogen, and is given only as a rough guide to the amount of structural material present; and there is a steady fall in sucrose and reducing sugars. On reirrigation the sugars (which we may regard as forming a metabolic pool) rise to normal levels; storage and structural compounds—as might be expected in leaves which expand, though not to normal size—rise, but do not attain normal levels. The decline observable in several constituents at the 32nd week is presumably a senescence phenomenon—it must be remembered that these leaves have survived for nearly twice their normal life-span. That the loss in sugars during the 'dry' period is due to respiration rather than translocation is suggested by the fact that such limited observations as we have made on leaves dried for 26 weeks show a fall in *all* constituents. Such leaves, incidentally, still retain the capacity to expand on reirrigation.

Two general conclusions emerge. First, the biochemical observations agree with the morphological and anatomical observations in being compatible with the view that the 'dry' leaves represent essentially normal leaves arrested at an early stage of development. Secondly, since there is no evidence whatsoever of synthetic activity, and at least indirect evidence of respiratory activity, the intercellular CO_2-content is likely to be permanently high; this will reinforce the effect of water-deficit in keeping the stomata closed.

5. TRANSPIRATION

We shall assume the reader to be familiar with our paper on transpiration and leaf water-content (Williams and Amer, 1957) and shall use the same symbols without further explanation. Hygen curves (Hygen, 1951) for two 4-week-dried leaves were obtained; Fig. 3*a* shows mean transpiration-rates at 10-minute intervals for the first 2 hours, and we note that one of the leaves (*A*) still shows the familiar stomatal opening on cutting the petiole. Fig. 3*b* shows the complete record (7 hours) plotted as transpiration-rate against water-content; idealized curves have been drawn in to simplify the picture, and there is a strong suggestion that the greater part, if not the whole, of the records lie within a linear falling-rate phase. If this is so, the Hygen equation applies and, if all the necessary ancillary data are available, the cuticular resistance R_2 can be calculated from the slope of the transpiration/time curve so long as n (in $n\rho_s$) remains unity. Unfortunately, our records are incomplete; in the thesis, however, calculations using likely values of ρ_s and ρ suggested that R_2 was of the order of 4·5 times the normal value.

A cruder, but perhaps more reliable, estimate can be obtained by other means. Consider the wilting 'wet' leaves which are recorded as Expts. 11–13 in Table IV[1] of our earlier paper; we will compare transpiration rates over the period ('second straight') 4–7 hours from the onset of wilting. The mean value for the 3 'wet' leaves is 8·5 mg./hr.; for the 2 'dry' leaves 2·5 mg./hr.—a factor of about 3·4. If leaf-areas are taken into account the corresponding values are $11{\cdot}8 \times 10^{-2}$ and $5{\cdot}8 \times 10^{-2}$ mg./cm.^{2}/hr.—a factor of 2. Now, the resistance values in the table show that R_2 is by far the most important, so that transpiration rates will be roughly inversely proportional to cuticular resistances, and R_2 for unit area for 'dry' leaves is roughly double that for 'wet'. The two values now obtained—4·5 and 2—are at least comparable in magnitude.

There is, however, another possibility. The cells are incompletely vacuolated, and the situation might be such that nowhere in the leaf is there a domain which could be regarded as fully saturated; this is equivalent to

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[1] We regret that there is a misprint in this table; for 'R_{3L}' read 'R_{2L}', and for 'R_2' (in line 7 of table) read 'R_3'.

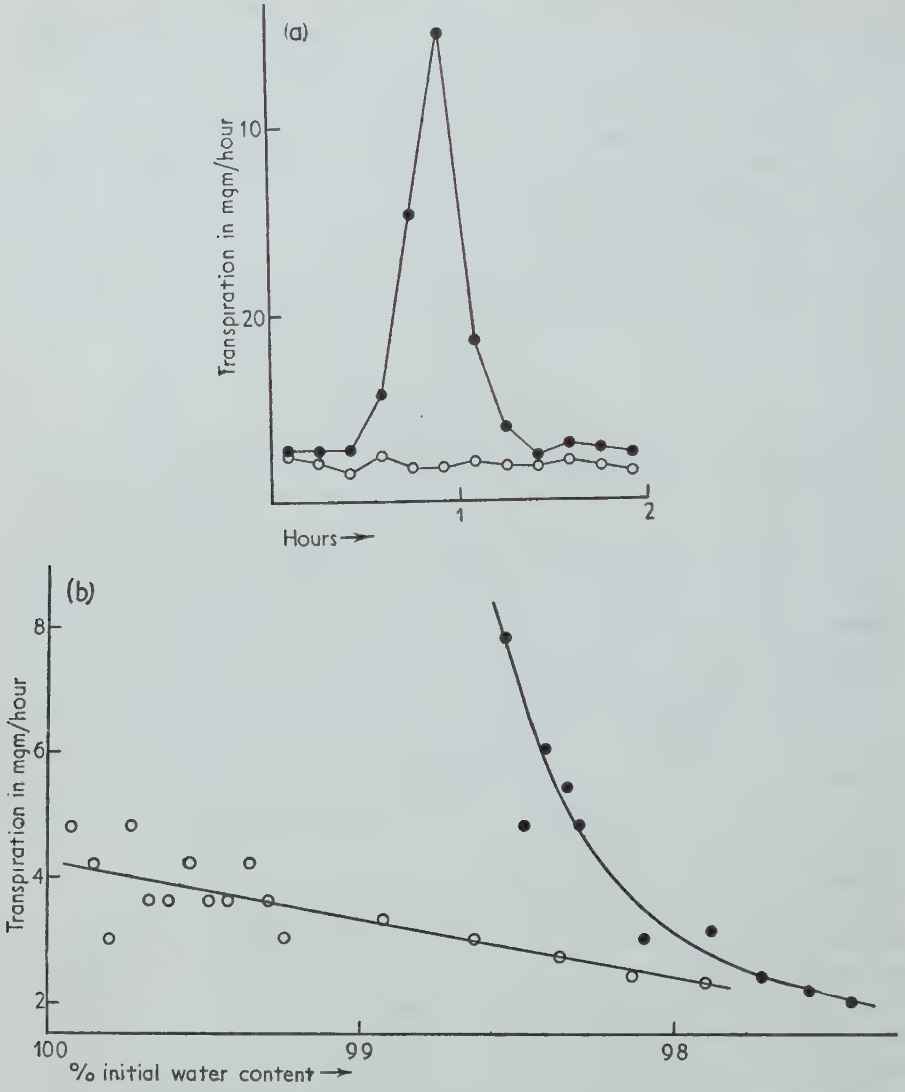

FIG. 3. Transpiration behaviour. Closed circles, leaf A; open circles, leaf B (for explanation see text).

putting $n<1$ in the Hygen equation. If, then, we assume that there is no difference in cuticular resistance, and if we take $\rho_s = 1{\cdot}5 \times 10^{-5}$ g./cm.^{3} and the external relative humidity as 60 per cent., we should obtain a value for n of about 0·8.

In order to distinguish between these two explanations of the reduced transpiration—which might of course be operating simultaneously—we had wondered whether differences in cuticular resistance might be accompanied by obvious differences in cuticle thickness. To this end, portions of upper and lower epidermis of both 'wet' and 'dry' leaves were embedded in methacrylate, sectioned, and examined under the electron microscope (Plate 2). The cuticle—which we take to be the clear, non-lamellar, superficial layer—is certainly no thicker in the 'dry' specimens; in fact, 'dry' epidermal walls are noticeably thinner as a whole than 'wet 'though they appear to us to be more compact. Clark and Levitt (1956) have observed that transpiration differences between normal and droughted leaves of *Soja* can be eliminated or even reversed by treatment of droughted leaves with alcohol; and, though they ascribe this effect to the lipid-dissolving properties of the alcohol, it is likely that the whole structure of the wall would be affected by such treatment. In this connexion, too, it should be noted that our specimens have, for embedding, been subjected to alcohol, 'cellosolve', and methacrylate monomer—all of which are to some extent fat-solvents.

Despite this inherent uncertainty as to the controlling factors, the most surprising feature of 'dry' leaf transpiration is its vigour—area for area, no less than half that of a normal leaf. The transpiration values, in fact, suggest that these leaves have no special adaptations—the necessarily high water-tension, acting through $n\rho_s$, would easily explain the modest reduction in water-loss. However, owing to the prompt shedding of mature leaves under droughted conditions, the total transpiring area of the plant is greatly reduced; and it is this, rather than any special mechanism, which reduces the overall water-loss.

6. CONCLUSIONS

So far as its leaves are concerned, *Pelargonium zonale* is a drought-*evading* plant. We have found no special anatomical modifications; no increase in sugars (cf. Clements, 1937*b*); no increase in hemicelluloses (cf. Clements, 1937*a*); no striking transpiration behaviour. The normal leaves are very sensitive to drought, and are quickly shed when water is withheld; but, since both production of leaves and individual leaf-development are slow, it is inevitable that one or two leaves are, as it were, caught in mid-expansion. The 'dry' leaves, then, seem to be essentially normal but arrested in an immature condition; photosynthesis is in abeyance, the stomata are closed, and the cells incompletely vacuolated. It is this latter condition which presumably explains their survival; the poorly vacuolated cells of many mosses and angiosperm seeds (Iljin, 1930), and of angiosperm apical meristems (Milthorpe, 1948), are similarly very resistant to drying since their imbibitional powers are so high. This accords well with the view that drought injury is mainly mechanical, brought about by excessive shrinkage of the cell—a view particularly associated with Iljin. There may well be some more special properties associated with the cells of the pith of *Pelargonium* stem, whose ability to withstand a ninefold reduction in volume is notorious (Iljin, 1930); but with these we have not been concerned. We see no reason to endow the leaves with any special drought-resisting properties whatever.

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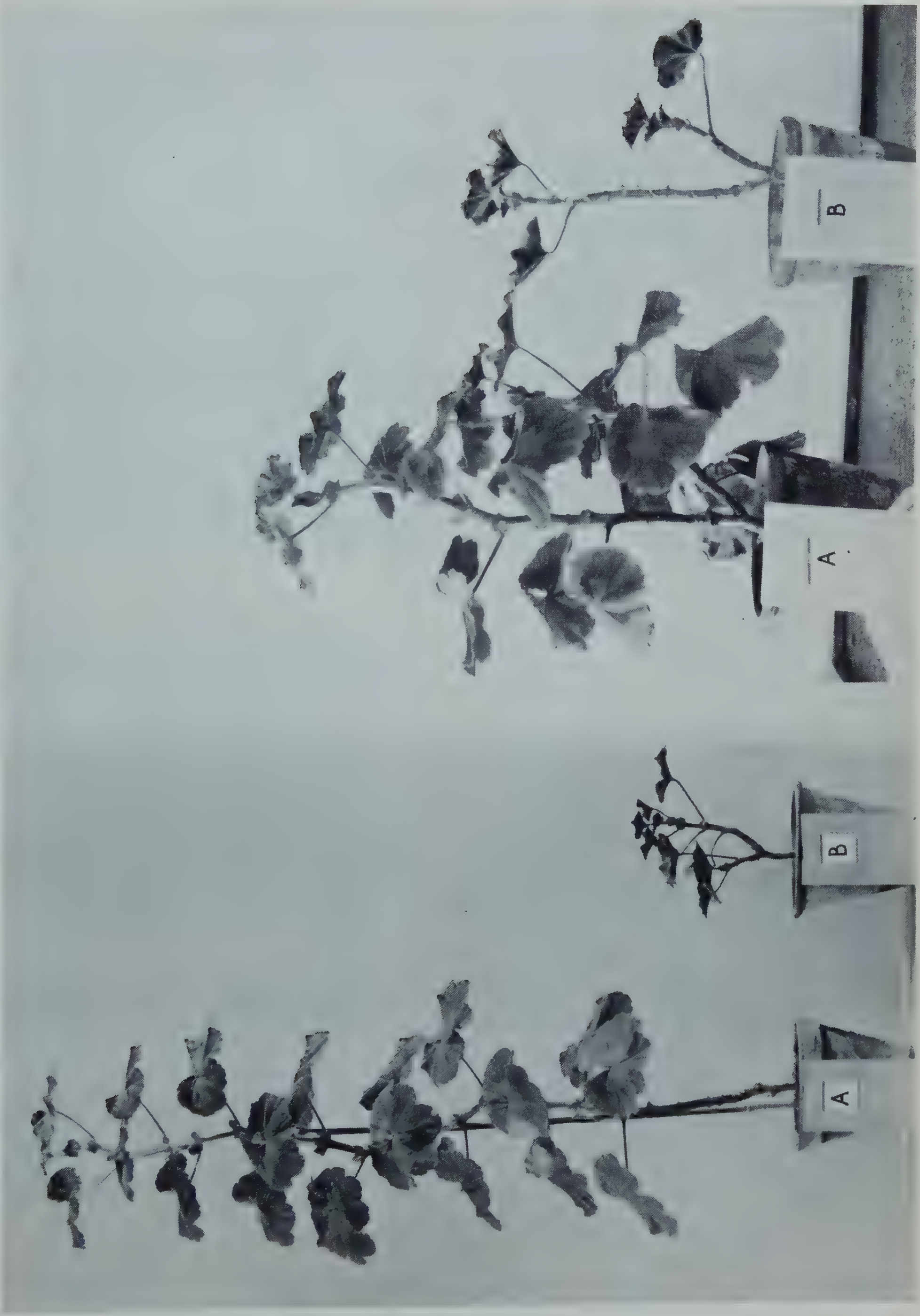

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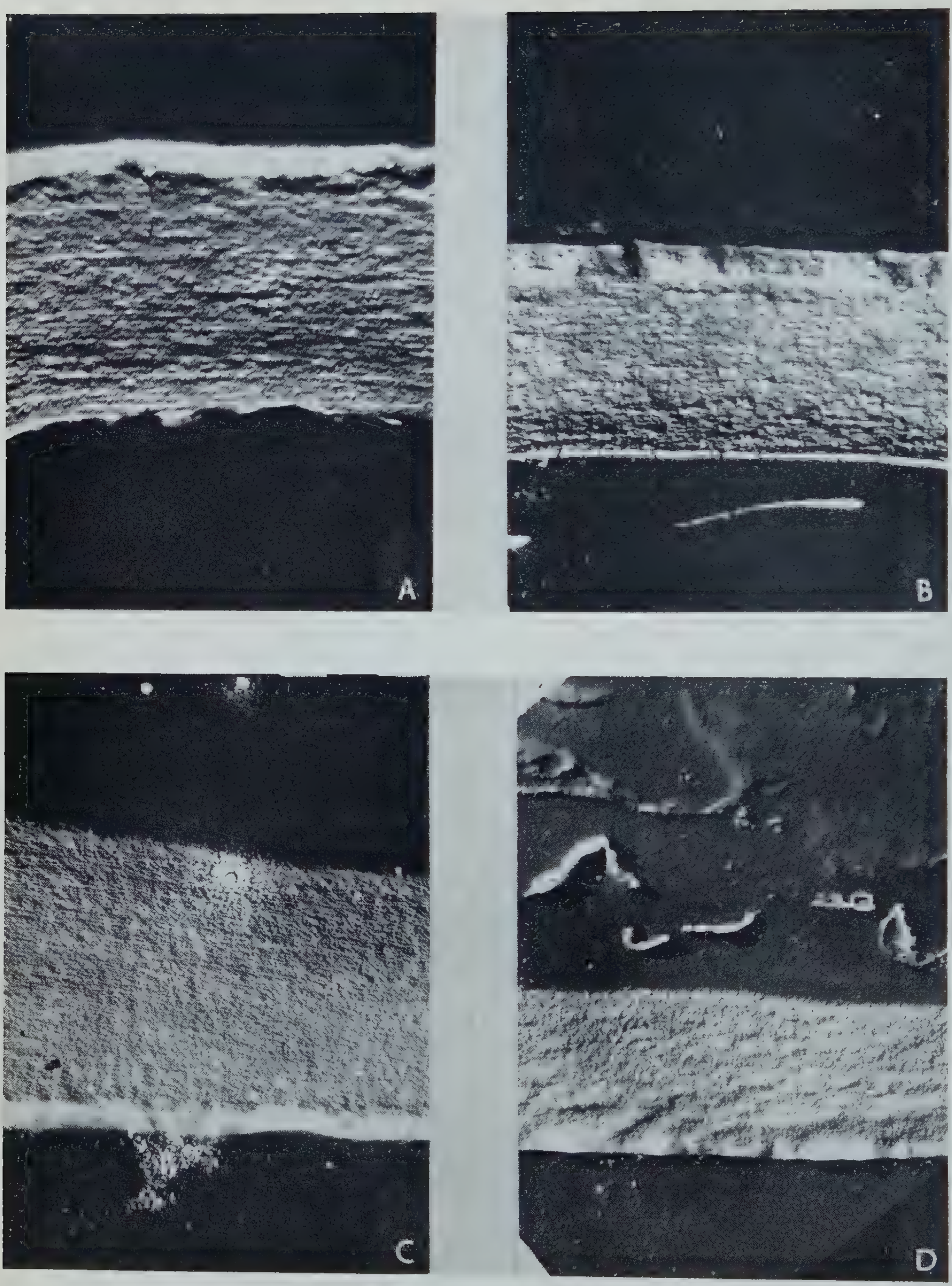

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LEGENDS FOR PLATES

PLATE 1

Effect of watering régime on general appearance of rooted *Pelargonium* cuttings: A, cuttings watered daily; B, cuttings of comparable age from which water has been withdrawn for 16 weeks.

PLATE 2

Electron micrographs of sections of wall of leaf epidermes. (*a*) upper, 'wet'; (*b*) upper, dry'; (*c*) lower, 'wet'; (*d*) lower, 'dry'. (All *c.* × 3450.)

Factors Affecting Uptake of Radioactive Phosphorus by Leaves and its Translocation to other Parts of the Plant

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With five Figures in the Text

Abstract

The rate of uptake of ^{32}P from labelled NaH_2PO_4 solutions sprayed on to one leaf of swedes (*Brassica napus*) or French beans (*Phaseolus vulgaris*) was rapid during the first few hours and fell to zero after 4 days. ^{32}P was detected in the root after 3 hours and continued to move out of the treated leaf for at least 6 days after application. A larger fraction of the applied ^{32}P was absorbed from repeated than from a single spraying.

Swedes absorbed more ^{32}P from a single application to the lower surface than to the upper surface of the leaf. Doubling the concentration of the spray caused a small increase in the percentage of applied ^{32}P that was absorbed. Absorption by French-bean leaves decreased slightly when the area sprayed with a constant amount of ^{32}P was doubled, and decreased with increasing age of leaf. Increasing the phosphorus supply to the roots of swedes affected neither the initial rate nor the total amount of ^{32}P uptake by the leaves but decreased the quantity of ^{32}P that was translocated out of the treated leaf.

Increasing the relative humidity of the air around the plants also increased ^{32}P uptake. Shading usually decreased uptake and always decreased translocation. Rewetting the leaf to which the ^{32}P had been applied, with water or sucrose solution, had variable effects. The significance of the results is discussed.

Introduction

FIELD and pot experiments have established that phosphorus is absorbed from solutions applied to leaves and the phosphorus so absorbed may increase growth of the plant (Thorne, 1954), but little is known about the factors that affect the rate of uptake and translocation of phosphorus from the leaf. The amount absorbed can be measured from the increase in phosphorus content of treated plants, but this increase must be large compared with the variation in content of untreated controls, and so numerous applications of solution to all the leaves of a plant are required. This type of experiment is thus not suitable for studying factors that may influence uptake, such as age of leaf, nutrient content of the plant, atmospheric humidity, and temperature, which vary between leaves and during the time that the leaf sprays are being applied.

If the phosphorus applied to the leaf is labelled with a radioactive tracer, very small amounts can be detected, giving a direct measure of uptake, as none is present in untreated plants. By this means uptake from solutions applied

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to single leaves can be measured in experiments lasting only a few days, during which time factors that may affect uptake can be controlled. In long-term experiments, an approximately constant fraction of the applied phosphorus is absorbed by the leaves; analysis of solutions resulting from washing the leaves showed consistently that about 90 per cent. of the phosphorus applied was absorbed (Thorne, 1957). But conditions that do not alter the total amount absorbed may affect the rate of uptake, and this can be tested in short-term experiments with tracers. As radioactive phosphorus applied to the leaf can be identified in all parts of the plant, uptake studies with tracers allow the effects of various factors on movement of phosphorus through the plant, as well as on uptake, to be studied.

To investigate some of the conditions controlling uptake of phosphorus by leaves and its translocation to the rest of the plant, phosphate solution labelled with radioactive phosphorus (^{32}P) was applied to one leaf of each of a number of plants. Plants were put in differing environments, or the treated leaf was sprayed with other solutions, and after some days the quantity of ^{32}P in various parts of the plant was estimated. Uptake by plants with differing previous treatments was also compared.

METHODS

All the experiments except Expt. 6 were done with swedes (*Brassica napus*, var. Purple King) or French beans (*Phaseolus vulgaris*, var. Canadian Wonder) grown singly in small glass pots, each holding about 250 g. of dry sand. Each plant was given 50 ml. of a complete nutrient solution (half-strength Hoagland) weekly. In Expt. 6, swedes were grown in 100 ml. conical flasks of Hoagland nutrient solution modified to contain 7·8 or 46·5 mg. P/litre. These solutions were renewed fortnightly. Treatments were applied when the swedes had about 5 leaves and the French beans two or three compound leaves.

Application and estimation of ^{32}P. Usually the solution applied to the leaves was 0·1 M. NaH_2PO_4 containing about 10 μC./ml. of ^{32}P, added as H_3PO_4, and 0·1 per cent. sulphonated lorol or Lissapol as a wetting agent. Depending on the size of the leaves in a particular experiment, 0·02–0·05 ml. of the solution was sprayed on a leaf with an aerograph air brush (Batt, Bennett, and Thomas, 1954). Care was taken to avoid run-off and the sprayed leaf was backed with filter paper to prevent contamination of the rest of the plant. The third leaf of swede plants having 5 leaves was usually chosen for treatment.

At the end of the absorption period the treated leaf was cut off at the base of the petiole and washed by dipping into a solution of 2 per cent. sodium phosphate containing 5 per cent. HCl and a wetting agent, rinsing in a jet of the acid phosphate solution, and finally rinsing in a jet of distilled water. The total washings from one leaf were made up to 100 ml. Tests showed that this procedure removed the ^{32}P on the outside of the leaf satisfactorily without leaching any from inside. Thus, solution from a further washing of the leaf contained negligible amounts of ^{32}P and, when leaves containing ^{32}P that had been absorbed by the roots were washed in this way, none was leached from them. The roots were washed free from sand, and the treated leaf, the rest of the tops, and the roots were separately digested with H_2SO_4 and HNO_3. The resulting solutions were each made up to 50 ml. and the ^{32}P determined with a Geiger-Müller counter using a 10 ml. M6 liquid counting tube. In a few experiments treated leaf, tops, and roots were combined before digestion.

Expression of results. The ^{32}P found in each part of the plant was expressed as a percentage of the total applied, i.e. the quantity of ^{32}P found in the whole plant plus that washed off the treated leaves. In this way comparisons can be made between the results of different experiments that would be impossible if the ^{32}P contents were given as counts-per-minute, because the specific activities of the solutions applied to the leaves varied between experiments. The quantity of ^{32}P recovered in the plant and on the surface of the treated leaf was a better measure of the amount applied than was the volume of solution put in the spray gun, because only about 80 per cent. of it was deposited on the leaf, the remainder being dispersed into the air as a fine mist.

Control of factors affecting absorption. In some experiments, the leaf to which ^{32}P had been applied was sprayed with other liquids during the absorption period; enough was applied to wet the leaf but avoiding run-off. The treated leaf was sprayed with water twice during a 3-day absorption period in Expt. 20, and nine times during 5 days with water or 1 per cent. HCl in Expt. 10. In Expts. 15 and 16 it was sprayed daily for 5 or 4 days respectively with water or 10 per cent. sucrose solution containing 0·025 per cent. sulphanilamide. Sucrose solution was also applied to the treated leaf 7 times in 6 days in Expt. 11, 7 times in 4 days in Expt. 17, and 4 times in 4 days in Expt. 18.

In other experiments the plants were enclosed in boxes measuring 18×30×12 inches with sides and tops made of transparent plastic; the boxes could accommodate up to 20 plants. A stream of air was passed through each box to ensure an adequate CO_2 supply. To study uptake in the dark one box was made light-tight by covering with black paper. In Expts. 12, 13, and 14 an attempt was made to vary the humidity inside the boxes; the incoming air was either dried by passage through a $CaCl_2$ drying tower and over petri-dishes placed between the plants containing silica gel renewed twice a day, or moistened by passage through two wash bottles of water and over damp filter paper on the bottom of the box. Because of transpiration from the plants, the air in the dry box was always damper than that outside in the glasshouse, but it was drier than the air in the damp box, which was often saturated, and water condensed on the walls of the box. Typical differences in humidity were those of Expt. 14 in which the average relative humidity during the absorption period, estimated from wet- and dry-bulb thermometer readings at 9.30 a.m. and 4.30 p.m., was 64 per cent. (at 23° C.) in the greenhouse, and 79 per cent. (at 25° C.) in the dry box. These correspond to water-vapour concentrations in the air of 12·8 and 17·8 g./m.^{3}, the value for saturated air at 25° C. being 22·8 g./m.^{3} Covering the plants increased temperature as well as humidity. In Expt. 19 uptake at 21–22° C. was compared with that at 13–14° C. using swede plants kept in growth chambers[1] for 3 days and given 8 hours of natural light daily.

All experiments were arranged in randomized blocks with 4–6 replicates. The plants were selected by eye for uniformity within blocks shortly before the ^{32}P was applied.

RESULTS

General features of uptake. The rate of phosphorus uptake from a single application of solution to one or more leaves of a plant was rapid during the first few hours and subsequently fell (Fig. 1). In the two long continued experiments uptake ceased after about 4 days. The initial rate of absorption and the total amount absorbed varied greatly between experiments. Uptake was less in experiments done in spring and autumn than in the summer (Fig. 2); absorption by plants under transparent covers is distinguished from that by uncovered ones in Fig. 2 because covering increased uptake (p. 388).

Most of the absorbed phosphorus was found initially in the leaf to which the solution had been applied (Fig. 5). ^{32}P was detected in the rest of the top and root after 3 hours in some experiments, but not until 1 day after application in others. Translocation from the treated leaf continued while the rate of uptake decreased, so that the ^{32}P content of the rest of the plant increased at the expense of the treated leaf.

In the experiments described below, only the ^{32}P contents of the whole plants are given unless treatments affected the distribution of ^{32}P within the plant. The amounts absorbed at different times after application are shown in the tables when treatments affected the initial rate of uptake. Otherwise the uptakes are those at the final harvest, or the means of the last 2 or 3 harvests when there was little or no increase in uptake during this period.

To contrast with absorption from a single application of phosphorus solution, Fig. 3 shows the course of uptake from repeated applications. Several leaves were sprayed, and therefore the ^{32}P in the treated leaves cannot be distinguished from that in the rest of the tops. The rate of uptake was initially much less than the rate of supply, but increased subsequently until after 2 weeks the absolute amount of ^{32}P within the plant represented a nearly constant proportion, about 75 per cent., of that applied.

Factors affecting uptake

(1) *Difference between leaf surfaces.* More ^{32}P was absorbed by the lower (abaxial) than by the upper (adaxial) surface of swede leaves (Table 1, Expts. 3 and 4). Distribution within the plant was the same for absorption by either surface. In all other experiments spray was applied to the upper surface of the leaf, because it was difficult to avoid run-off from the underside.

(2) *Concentration of spray.* The effect of applying equal volumes of sprays differing in concentration was tested. In Expts. 4 and 6 the concentrated

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[1] Dr. W. W. Schwabe of the Imperial College of Science and Technology kindly provided space in his controlled-environment chambers.

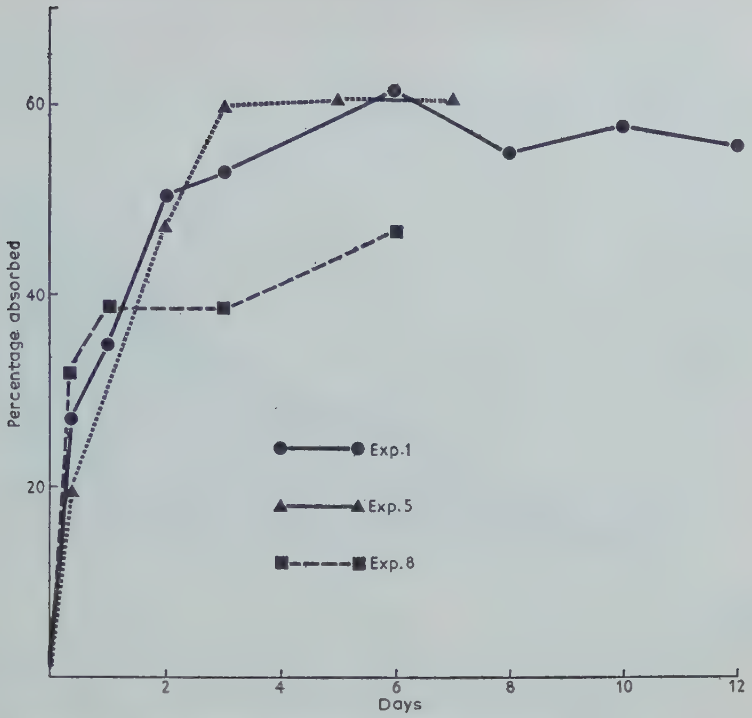

FIG. 1. Uptake of ^{32}P from single applications of sodium phosphate solution to leaves. Means of all treatments, Expts. 1, 5, and 8.

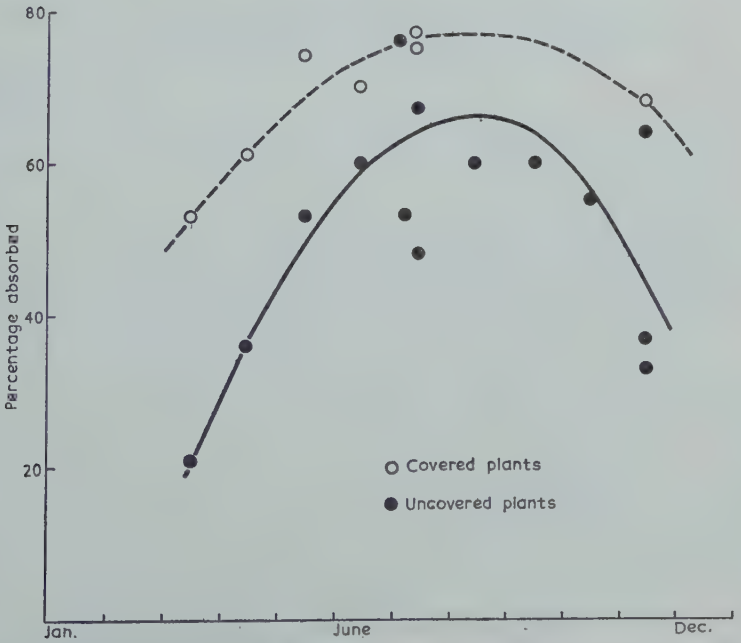

FIG. 2. Uptake of ^{32}P by leaves at the end of experiments done at various times of year.

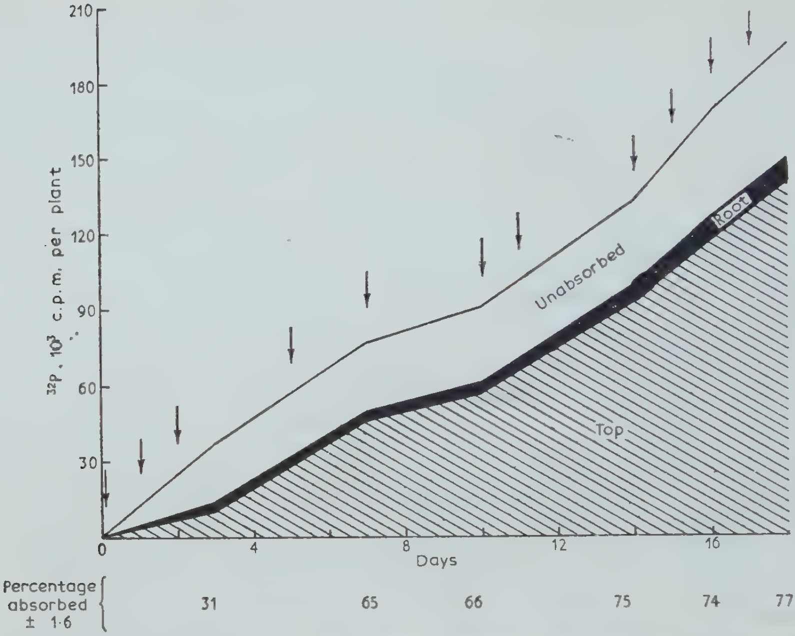

FIG. 3. Uptake of ^{32}P from repeated applications of solution, at times shown by the arrows, to leaves of swedes. Expt. 2.

TABLE 1

Uptake of ^{32}P by Upper and Lower Surfaces of Swede Leaves from Sprays of two Phosphorus Concentrations

Expt.	Treatment		Percentage absorbed
3	Upper surface sprayed		8
	Lower ,, ,,		33
	S.E.		3·1
4	Means of both sprays	Upper surface sprayed	48
		Lower ,, ,,	57
	Means of both surfaces	Dilute solution (1·55 mg.P/ml.)	47
		Concentrated solution (3·10 mg.P/ml.)	58
		S.E.	1·8

spray had twice the phosphorus content of the dilute spray and therefore supplied twice the amount of phosphorus, and in Expt. 5 sprays of three different concentrations were tested. The percentage of the ^{32}P supplied that was absorbed in 4 days was greater from the concentrated than from the dilute spray in Expt. 4 (Table 1), and in Expt. 5 (Fig. 4) there was relatively less absorption from the most dilute spray than from the other two. In Expt. 6 doubling the concentration of the spray increased the initial rate of uptake but had no significant effect on the final percentage absorbed (Fig. 4).

Similar amounts of ^{32}P were applied to French beans (Table 2, Expts. 7

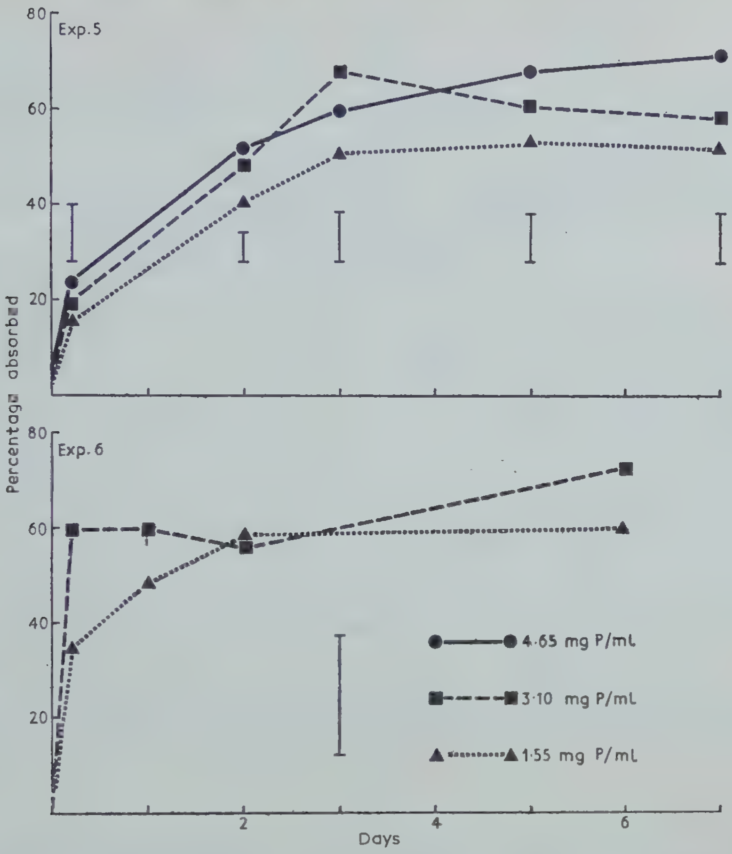

FIG. 4. Uptake of ^{32}P by leaves of swedes from solutions of various concentrations. Least significant differences ($P=0·05$) are shown for each harvest in Expt. 5, and a single value appropriate to all harvests is shown in Expt. 6.

and 8) as dilute or concentrated sprays. The volume of dilute solution applied was twice that of concentrated solution and it was sprayed on twice the area of leaf. Uptake from the concentrated spray appeared to be initially faster than from the dilute spray in Expt. 7; it was greater from the concentrated than from the dilute spray after 3 but not after 6 days. But in Expt. 8 there were similar small differences in the amounts absorbed at all harvests.

The distribution of ^{32}P within the plant was independent of the concentration of the spray.

(3) *Age of treated leaf.* In the two experiments with French beans more ^{32}P was absorbed by young than by old leaves (Table 2). Most of the extra phosphorus absorbed by the younger leaves moved out of them, so that similar amounts of ^{32}P remained in both young and old leaves.

(4) *Phosphorus supply to roots.* Phosphorus supply to the roots of swedes affected neither the total amount of ^{32}P absorbed by the leaf nor the initial rate of uptake, but with low phosphorus supply to the roots more phosphorus

TABLE 2

Percentage Absorption of ^{32}P from 0·10 ml. of Dilute Solution (1·55 mg. P/ml.) or 0·05 ml. of Concentrated Solution (3·10 mg. P/ml.) Applied to Young or Old Leaves of French Beans

Expt. 7: young leaf = 3rd; old leaf = 1st or 2nd (primary); Expt. 8: young leaf = 4th; old leaf = 3rd. Dilute solution applied to both primary leaves or two opposite leaflets of compound leaf, concentrated solution applied to one primary leaf or leaflet.

	Experiment 7				Experiment 8				
	3 days	6 days	Means of 3 and 6 days		1 day	3 days	6 days	Means of 3 and 6 days	
	Whole plant		Treated leaf	Whole plant	Whole plant			Treated leaf	Whole plant
Means of both leaves:									
^{32}P applied as 0·10 ml. dilute solution	39	50	25	44	42	36	43	21	40
^{32}P applied as 0·05 ml. concentrated solution	48	52	29	50	37	42	51	29	46
Means of both concentrations:									
^{32}P applied to younger leaf	51	60	26	56	43	46	53	27	50
^{32}P applied to older leaf	35	42	27	39	35	32	41	23	36
S.E.	2·5		1·4	1·8	2·5			1·3	1·8

moved out of the sprayed leaf than with high phosphorus supply (Fig. 5). In this experiment the mean contents of plants with low and high supplies of phosphorus were 2·2 and 3·8 mg. P per plant respectively.

(5) *Effect of covering and shading plants.* In a preliminary experiment intended to test the effect of decreased carbohydrate content of the leaf on ^{32}P uptake, plants placed under a cover to exclude light absorbed more ^{32}P than uncovered ones (Table 3, Expt. 9). It was found subsequently that enclosing plants under either opaque or transparent covers increased ^{32}P absorption (Table 3, Expt. 10; Tables 4, 6, and 7). Enclosing the plants in a box altered a number of factors that might affect uptake; it increased both humidity and temperature. Uptake of ^{32}P by plants kept in a damp atmosphere was greater than in a drier one (Table 4). Uptake by unenclosed plants was always less than by covered ones, probably because it was impossible to keep the air in the box as dry as in the greenhouse (p. 383). Water content of the laminae was decreased by drying the air in Expt. 12 (Table 4) but was unaffected in Expt. 13 possibly because the plants were harvested in the early morning. Expt. 12 was harvested in the afternoon when the plants had been transpiring actively, and when differences in atmospheric humidity would probably have their greatest effect.

A temperature difference of 8° C. during the absorption period did not affect uptake (Table 5), although an increase with temperature could have been masked by a decrease with lower humidity, which was not controlled. However, it is unlikely that the smaller temperature increase caused by covering (about 3° C.) had any effect on uptake.

If more humid conditions increased uptake by causing the spray to dry more slowly, rewetting the leaf with water after the spray had dried should

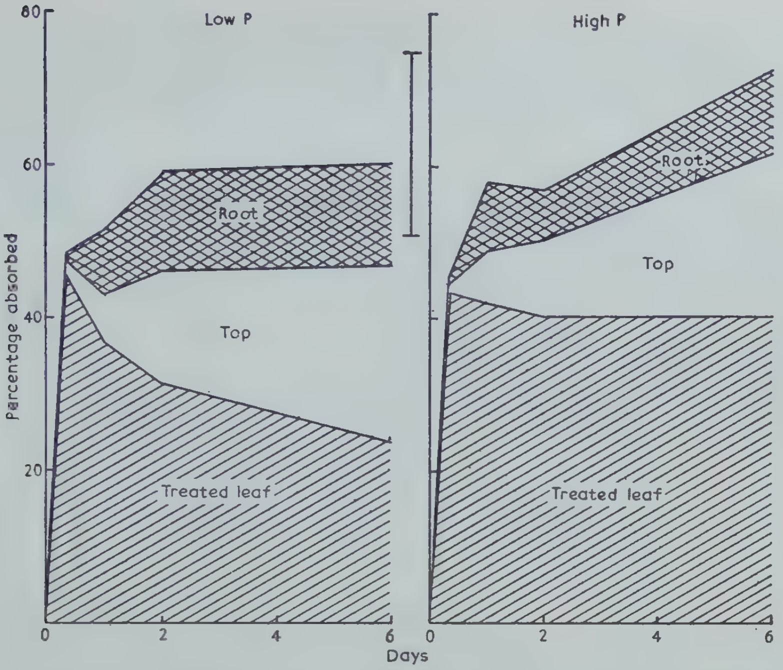

FIG. 5. Uptake of ^{32}P by leaves of swedes having low or high supply of phosphorus to the roots, and the distribution of ^{32}P within the plant. The least significant difference ($P = 0{\cdot}05$) for ^{32}P uptake in the whole plant after 6 days is shown by the vertical line between the two parts of the figure.

TABLE 3

Effect of Covering, Shading, or Spraying Treated Leaf with Sucrose, Water, or HCl, on Percentage Uptake of ^{32}P by Leaves and on the Amount of ^{32}P Remaining in the Treated Leaf of Swedes

Expt.	Treatment	Percentage uptake by whole plant	Percentage of total uptake remaining in treated leaf
9	Covered in dark	53	87
	Uncovered	17	60
	S.E.	3·1	3·5
10	Covered in dark	59	64
	Covered in light	62	32
	Uncovered	36	49
	Uncovered, treated leaf sprayed with water	44	53
	,, ,, ,, 1% HCl	35	44
	S.E.	3·2	2·9
11	Covered in dark	36	73
	Covered in dark, treated leaf sprayed with sucrose	55	30
	Covered in light	60	41
	S.E.	6·0	6·8
20	Uncovered	59	
	Uncovered, treated leaf sprayed with water	60	
	S.E.	2·5	

TABLE 4

Effect of Humidity on Uptake of ^{32}P by Leaves and on Dry Matter per cent. of Fresh Weight of the Laminae of Swedes

Treatment	Percentage absorbed Expt. 12	Percentage absorbed Expt. 13	Percentage absorbed Expt. 14	Dry matter per cent. of fresh weight Expt. 12
Covered, damp atmosphere	74	65	59	14·4
Covered, dry atmosphere	67	58	45	15·2
Uncovered	53	—	27	15·5
S.E.	2·5	1·7	3·0	0·28

TABLE 5

Expt. 19. Uptake of ^{32}P by Leaves of Swedes at 14° C. and 22° C.

	Percentage absorbed	Relative humidity
21–22° C.	45	75%
13–14° C.	44	85%
S.E.	3·7	

TABLE 6

Effect of Subsequently Spraying the Treated Leaf with Sucrose or Water on Percentage Uptake of ^{32}P by Leaves of Swede Plants that were Covered in the Dark (D), Covered in the Light (L), or Uncovered (U)

		Experiment 15					Experiment 16	
		Treated leaf	Top	Root	Whole plant	Percentage in treated leaf	Whole plant	Percentage in treated leaf
Treated leaf, not sprayed	D	11	36	4	51	22	7	98
	L	26	34	14	74	34	19	86
	U	11	31	16	59	19	3	82
Treated leaf, sprayed with sucrose	D	21	47	5	73	30	42	95
	L	34	43	12	88	39	67	67
	U	26	31	13	69	36	37	77
Treated leaf, sprayed with water	D	16	56	5	76	21	31	98
	L	31	33	14	77	40	68	78
	U	20	34	19	73	28	7	75
S.E.		4·0	4·4	1·8	3·6	5·4	4·7	2·5
Means of all sprays	D	16	46	4	67	24	27	97
	L	30	37	13	80	38	51	77
	U	19	32	16	67	28	16	78
Means of D, L, and U	Unsprayed	16	34	12	61	25	10	89
	Sprayed with sucrose	27	40	10	77	35	49	80
	Sprayed with water	22	41	12	75	30	35	84
S.E.		2·3	2·5	1·0	2·1	3·1	2·7	1·5

also increase uptake. Rewetting the treated leaf of uncovered plants had no effect on uptake in Expts. 10, 16, or 20 (Tables 3 and 6) but increased it in Expt. 15 (Table 6). Dilute HCl, in which phosphates are more soluble than in water, had no effect (Table 3, Expt. 10). Spraying the treated leaf of covered plants with water increased uptake in both light and dark in Expt. 16 but only in the dark in Expt. 15 (Table 6). The reasons for these differences between experiments are not known.

The effect of light on ^{32}P uptake was studied by comparing plants enclosed under transparent covers with those under opaque ones. Shading decreased uptake in Expts. 11, 15, 16, and 18 (Tables, 3, 6, and 7), and had no effect in Expts. 10 and 17 (Tables 3 and 7). In all experiments except Expt. 15, shading decreased translocation from the treated leaf to the rest of the top and the root; i.e. the percentage of the absorbed ^{32}P that occurred in the tops and roots was always greater in illuminated than in shaded plants. In Expt. 15 shading decreased translocation to the root but apparently increased it to the top (Table 6).

TABLE 7

Effect of Spraying Treated Leaf with Sucrose on Percentage Uptake of ^{32}P by Leaves of French Bean or Swede Plants that were Covered in the Dark (D), Covered in the Light (L), or Uncovered (U)

		Expt. 17. French beans		Expt. 18. Swedes	
		Whole plant	Percentage in treated leaf	Whole plant	Percentage in treated leaf
Treated leaf not sprayed	D	77	80	23	78
	L	75	59	77	56
	U	67	61	15	66
Treated leaf sprayed with sucrose	D	68	81	39	77
	L	75	48	91	52
	U	54	55	45	57
S.E.		3·9	7·2	4·5	6·3
Means of not sprayed and sprayed with sucrose	D	73	81	31	78
	L	75	53	84	54
	U	61	58	30	61
S.E.		2·7	5·1	3·2	4·4
Means of D, L, and U	Unsprayed	73	66	38	67
	Sprayed with sucrose	66	61	58	62
S.E.		2·2	4·1	2·6	3·6

If the effect of shading on ^{32}P absorption and translocation is a result of a reduction in the carbohydrate content of the plant, sucrose applied to the treated leaf should counteract the effect of darkness, because sucrose is absorbed by leaves (Hull, 1952). This was tested in five experiments. In Expt. 11 (Table 3) sucrose increased uptake and translocation to the rest of the plant in the dark, but the effect of sucrose on unshaded plants was not tested. In Expts. 15, 16, and 18 (Tables 6 and 7) sucrose increased uptake in all conditions (covered and shaded, covered and unshaded, uncovered). When applied to the treated leaf of French beans (Expt. 17; Table 5), sucrose significantly decreased uptake by uncovered plants, caused a smaller but non-significant decrease in uptake by shaded plants, and had no effect on covered illuminated plants. The only effects of sucrose on translocation were that movement of ^{32}P from the treated leaf of illuminated covered plants was increased in Expt. 16 and that from uncovered plants was decreased in Expt. 15.

These varied effects of sucrose on ^{32}P uptake were not clarified when spraying the treated leaf with sucrose was compared with spraying it with water (Table 6). In the dark, spraying with water increased uptake in the same way as spraying with sucrose. In the light, water increased uptake by uncovered plants in Expt. 15 and by covered plants in Expt. 16.

DISCUSSION

The rate of uptake of ^{32}P from a single application of solution to a leaf was rapid at first and gradually slowed down to zero after about 4 days. Uptake of nitrogen, phosphorus, and magnesium by apple leaves (Fisher and Walker, 1955), and of a range of nutrients by bean leaves (Bukovac and Wittwer, 1957) has been found to follow a similar course, but the initial rate of uptake and the total amount absorbed varied with both the nutrient and the plant species. As in the present experiments with swedes and French beans, uptake by apple leaves reached a maximum 2 to 8 days after application; the maximum percentage of nutrient applied that was absorbed was 90 per cent. for N, 50 per cent. for P, and 20 per cent. for Mg. However, Bukovac and Wittwer (1957) found slight absorption still continuing 8 days after treatment. The percentage absorption of phosphorus by swedes and French beans was less than the almost complete uptake of nitrogen and potassium reported by Cook and Boynton (1952), and Thorne (1957). A greater fraction of applied phosphorus appears to be absorbed from repeated applications (Fig. 3) than from a single application, and the uptake may reach 90 per cent. after 4 weeks of daily application (Thorne, 1957).

The rapid movement of ^{32}P from leaves to roots demonstrated in various species by autoradiographs (Wittwer and Lundahl, 1951) was also found in swedes; ^{32}P was detected in the root 3 hours after application to the leaves. Bukovac and Wittwer (1957) found similar movement of sodium and potassium to the roots of French beans, but other nutrients such as calcium, iron, and manganese did not move out of the treated leaf.

The large variation between experiments suggests that uptake of ^{32}P by leaves and its subsequent movement to the rest of the plant depend on a number of factors that probably interact. The effects of some of these factors were demonstrated in experiments involving comparisons of two or more of them. Uptake by the lower surface of swede leaves was greater than from the upper surface, probably because of the thinner cuticle; there are similar numbers of stomata on both surfaces. Moreover, the presence of stomata is not an important factor in controlling nutrient uptake by leaves; the hypostomatous apple leaf absorbs nitrogen through both surfaces (Rodney, 1952; Cook and Boynton, 1952) and Gustafson (1957) showed that the relative uptakes of ^{60}Co by the two surfaces of leaves of several species were not related to the numbers of stomata. Many species absorb nutrients better through the lower surface, but beans absorbed more ^{32}P through the upper surface (Teubner, Wittwer, Long, and Tukey, 1957).

Phosphorus uptake by young French-bean leaves was greater than by older ones. Similarly, uptake of urea (Cook and Boynton, 1952) and phosphorus (Fisher and Walker, 1955) decreased with age of apple leaves. The recovery of ^{32}P in the untreated part of the plant after 24 hours from an application to the first trifoliate leaf of French bean was less than from the primary leaf, and still less from the second trifoliate leaf (Koontz and Biddulph, 1957).

Increasing the concentration of the spray had small and inconsistent effects on uptake either when the area treated or when the amount of phosphorus applied per unit area of leaf was held constant. In both cases increasing the concentration of the spray would increase the concentration gradient between the spray deposit and the absorbing cells. Uptake was slightly greater from the more concentrated spray in three out of five experiments. Neither Bukovac and Wittwer (1957) nor Koontz and Biddulph (1957) obtained greater uptake by treating a larger leaf surface. The latter authors found that raising the nutrient concentration in a solution on the leaf up to 0·01 M. improved uptake, but a further increase to concentrations in the range used in the present experiments had no effect. Uptake of zinc by citrus from a drop of solution was decreased by spreading the drop over the whole leaf surface (Wallihan and Heymann-Herschberg, 1956).

Increasing the phosphorus supply to the roots had no effect on the initial rate or total amount of uptake by leaves, but more phosphorus moved out of the treated leaf when the phosphorus supply to the root was low than when it was high. Similar results have been obtained with *Chrysanthemum* (Asen, Wittwer, and Teubner, 1954) and sugar beet (Thorne, 1957) but the percentage recovery in the untreated parts of phosphorus-deficient French beans was less than from plants with a normal phosphorus supply to the roots (Koontz and Biddulph, 1957). Increased accumulation in the roots of plants having a low phosphorus supply is not specific to phosphorus absorbed via the leaves, as it also occurs with root-absorbed phosphorus (Thorne, 1957).

Covering plants with transparent or opaque covers increased phosphorus uptake by the leaves. That the cause of this increase was the high humidity under the covers is clear from the experiments in which the humidity was deliberately varied. Differences in humidity are probably an important cause of variation in uptake between experiments. Uptakes by uncovered plants in Expts. 14, 15, and 18 were in the same order as both the mean relative and absolute humidities during the absorption period:

Expt.	*Percentage uptake*	*Relative humidity*	*g.H_2O/m^3 air*
18	15	54	10·8
14	27	64	12·8
15	59	82	13·1

How atmospheric humidity influences uptake is not clear. It presumably causes the spray to dry more slowly, and so should increase uptake if absorption occurs only before the spray has dried on the leaf. But the results show that uptake continued for several days after application, i.e. after the spray had dried. If absorption is slower after drying of the spray, increasing humidity could increase uptake by lengthening the period of rapid absorption while the spray was drying, so that high humidity would increase the total amount absorbed mainly by increasing the initial rate of uptake. Successive harvests were made in only two of the experiments that included humidity comparisons or tested the effect of covers. In one of these, maximum uptake apparently occurred by the first harvest, and in the other covering the plants or increasing the humidity increased the amount of phosphorus absorbed in similar proportions at each harvest. If high humidity acts by delaying the drying of the spray, rewetting the treated leaf should increase uptake, especially in dry atmospheric conditions. However, the results show, on the contrary, that rewetting was effective only when the humidity was high, i.e. on covered plants (Expts. 15 and 16), and in the one experiment (Expt. 15) out of four on uncovered plants when the humidity was exceptionally high. It seems likely, therefore, that high humidity influenced uptake not by maintaining a film of water on the leaf for a longer time, but by affecting the absorbing cells and increasing their water content (Table 4). As already noted (p. 388), humidity probably affected the water content only during the day and therefore should affect uptake only during the day. A study of the effects of humidity on uptake in the light and in the dark might determine whether water content of the absorbing cells is a factor controlling uptake. The effect of humidity on nutrient absorption by leaves has not previously received much attention, though humidity has been controlled in some investigations of other factors (Koontz and Biddulph, 1957). Although it apparently does not account for the effect of high humidity, slower drying of the spray is associated with increased nutrient uptake. Apple leaves absorbed more magnesium when sprayed in the evening than when sprayed in the afternoon, probably because of the higher humidity (Oland and Opland, 1956), and Cook and Boynton (1952) obtained greater uptake of urea by apple leaves in conditions that favour slow drying of the spray, such as low temperature and high relative humidity. Phosphorus absorption by bean leaves from several sodium and potassium compounds was inversely related to the rates of drying of the sprays, and addition of glycerine to KH_2PO_4 solution, which delayed the rate of drying, also increased phosphorus-uptake (Koontz and Biddulph, 1957). Phosphorus uptake by apple leaves was increased by adding glycerine to the spray and by rewetting the leaf with water three times during the 24-hour absorption period (Fisher and Walker, 1955). On the other hand, magnesium uptake by apples was not increased by rewetting (Oland and Opland, 1956). The present experiments showed that rewetting does not invariably increase nutrient uptake, and the differences between experiments may be due to variation in other factors, including relative humidity, that affect the response to rewetting.

Effects of darkness on nutrient absorption by leaves must be distinguished from the effects of covering plants that are apparent both in light and dark.

This was achieved in the present experiments by comparing plants enclosed under transparent or opaque covers. In four out of six experiments shading decreased phosphorus uptake by covered plants, and in all six shading decreased translocation from the treated leaf to the rest of the plant. Light had similar effects on the absorption of other elements. Uptake of ^{60}Co by bean leaves, and translocation from the treated leaf to the rest of the plant, increased with increasing light intensity; in complete darkness there was only slight uptake and negligible translocation (Gustafson, 1956; Gustafson and Schlessinger, 1956). Bean and tomato leaves absorbed more radioactive phosphorus, potassium, and rubidium in 8 hours following application in the morning than in the evening (Teubner *et al.*, 1957).

The smaller uptake and translocation of nutrients in the dark than in the light may result from a reduction in the carbohydrate content of the leaf. Both uptake and translocation of ^{60}Co decreased when the carbohydrate content of the leaf was reduced by darkening the plant before or during absorption. Uptake and, especially, translocation in the dark were increased by adding sucrose or fructose to the ^{60}Co solution (Gustafson, 1956). ^{32}P uptake by wheat leaves was also increased by adding sugars to the phosphate solution, fructose and glucose being more effective than maltose and galactose (Yatazawa and Tai, 1953). Autoradiographs and ringing experiments show that ^{32}P moves in the phloem in the same direction as carbohydrate (Colwell, 1942; Biddulph, 1956). An association between carbohydrate metabolism and phosphorus transport is indicated by the identification in sugar phosphates extracted from the stem of *Brassica chinensis* of ^{32}P previously applied to the leaves (Yatazawa and Higashino, 1953). But several metabolic inhibitors had no effect on uptake and transport of ^{32}P from bean leaves (Kendal, 1955), and light and added sugars increased uptake and translocation of cobalt which has not the close association with carbohydrate metabolism that phosphorus has. Both these results indicate that phosphorus uptake and transport are not specially associated with carbohydrate metabolism.

In the present experiments, sucrose apparently did not increase phosphorus uptake by leaves by overcoming a deficiency of carbohydrate caused by shading, because it increased uptake equally in the light and dark (Expts. 15, 16, and 18). All the plants of Expt. 18, which was done in the short days and low light intensity of December, may have been deficient in carbohydrate throughout the absorption period, and hence in a condition to respond to added sucrose, but the other two experiments were done in August and September when the illuminated plants should have quickly recovered from the 2-day pretreatment in the dark. Spraying the treated leaf with sucrose solution or adding sucrose to the ^{32}P solution could increase uptake other than via the carbohydrate metabolism of the plant, e.g. by redissolving the ^{32}P, or delaying drying of the spray in the same way as glycerol (p. 394), or as the addition of molasses delayed the drying and increased the effectiveness of DNOC sprays (Fogg, 1948), and Expts. 15 and 16 provide evidence that sucrose acted in this manner. Uptake in Expt. 15 was increased equally by rewetting the treated leaf with water or sucrose, but in Expt. 16 sucrose had the larger effect, possibly because there was also a metabolic effect as this experiment was done in conditions unfavourable for assimilation. However, redissolving the spray deposit on the leaf does not always increase uptake (p. 390) and so sucrose and water sprays may be acting directly on the absorbing cells by increasing their water content or changing their permeability to phosphate.

Although sucrose usually increased phosphorus absorption by leaves, it caused a small decrease in uptake by leaves of French beans in Expt. 17. Teubner *et al.* (1957) found a similar depression, though it only lasted a few hours and was less pronounced in the dark than in the light. It is not clear whether French beans differ from swedes in their response to sucrose or whether some other factor differed between the experiments. Sucrose usually decreases the uptake of urea by several species (Cook and Boynton, 1952; Volk and McAuliffe, 1954).

It was not always easy to distinguish effects on uptake from those on translocation. In the present experiments translocation was measured by change in the percentage of absorbed phosphorus that remained in the treated leaf. A factor that altered this without altering the total amount absorbed clearly had a direct effect on translocation, e.g. shading in Expts. 10 and 17, which decreased translocation without change in total uptake. But when the amount absorbed and the percentage translocated increased simultaneously, the same factor could have affected both the processes of absorption and transport, or increased translocation might have resulted from the increased concentration in the treated leaf. Such a dependence of translocation on the amount absorbed might account for the greater translocation from treated leaves of covered plants than of uncovered ones in Expt. 10, where covering caused a large increase in uptake. Covering the plants or raising the humidity did not increase translocation in other experiments. Decreased translocation in the dark was probably a direct effect on the process of transport itself, as it occurred when uptake was either decreased or unaltered. The movement out of leaves of auxin and insecticides, as well as of nutrients, depends on an adequate supply of carbohydrate to the leaf (van Overbeek, 1956). In the experiments described in this paper, unabsorbed nutrient on the surface of a leaf was removed by washing, and the whole leaf was then used to estimate the amount of nutrient absorbed, but other workers have discarded the treated part of the plant to avoid contamination from unabsorbed nutrient, and so absorbed nutrient in tissues below the treated surface was not included in the estimated uptake. In such experiments uptake was estimated as the amount translocated from the site of application. When a small area of leaf tissue was treated and removed (Gustafson, 1956; Bukovac and Wittwer, 1957), translocation to the rest of the plant could be distinguished from that to the rest of the treated leaf, but when a complete leaf was treated (Koontz and Biddulph, 1957) uptake could not be distinguished from translocation, and was underestimated by the amount that remained in the sprayed leaf, which experiments on washed leaves showed might be over half the total absorbed.

The variability of the results discussed in this paper shows that many more experiments must be done before the mechanism of nutrient uptake by leaves is understood. The problem is even more complicated than that of absorption by roots, which are continually immersed in solution, so that the concentration of nutrient and other external conditions are uniform and more easily controlled. The difference in concentration between the nutrient solution on a leaf and the absorbing cells, the degree of contact between the solution and the leaf surface, the physiological state of the leaf and the physical state of the solution—these are all dependent on external conditions; consequently, in order to distinguish the internal and external factors that control the rate of absorption by leaves, and to measure their effects, it seems essential to conduct experiments on uniform plant material in controlled environments.

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Fungal Associations

III. The Role of Pectic Enzymes on the Synergistic Relation between *Rhizoctonia solani* Kühn and *Fusarium solani* Snyder and Hansen, in the Rotting of Potato Tubers

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With four Figures in the Text

ABSTRACT

The greatest activity of protopectinase obtained from the growth of *Rhizoctonia solani* and *Fusarium solani* on autoclaved potato plugs occurred at pH 6·5, and greatest activity of the 'loss of viscosity' enzyme was found at 6·5 for *Rhizoctonia*, and between 6·5 and 8·3 for *Fusarium*. Protopectinase enzyme obtained from double infections of the *Fusarium* spp. with *Rhizoctonia*, or by mixing the enzymes of individual *Fusarium* spp. with *Rhizoctonia* enzyme, were more active than the enzymes from single inoculations. *Cylindrocarpon radicicola* enzyme was more active when obtained from a pure culture than from double infection. Similarly, mixing this enzyme with the enzyme of *Rhizoctonia* reduced its activity. The evidence indicated that the protopectinase of *Rhizoctonia* was similar to that of *Cylindrocarpon* and differed from that of the *Fusarium* spp.

Using paper partition chromatography, two bands from *Rhizoctonia* crude enzyme had a stimulatory effect on *Fusarium* enzyme, while only one band from *Fusarium* enzyme stimulated *Rhizoctonia* enzyme.

The purified enzyme of *Rhizoctonia* degraded pectin to galacturonic acid. *Fusarium* pure enzyme degraded pectin to an intermediate stage. A mixture of the two enzymes degraded pectin to galacturonic acid, without the intermediate stage formed by *Fusarium* alone being detected.

The role played by pectic enzymes upon the synergistic relation of *Rhizoctonia solani* and *Fusarium solani* on rotting potato tubers is discussed.

INTRODUCTION

IN the second paper of this series (Elarosi, 1957*b*) feeble growth of *Rhizoctonia solani* was observed on a medium containing pectin as the sole carbon-source, while good growth of *Fusarium solani* was obtained on the same medium. Further, the rate of growth of *Rhizoctonia* on pectin medium was stimulated in the vicinity of a *Fusarium* colony. Moreover, the presence of pectin medium used by *Fusarium* stimulated the growth of *Rhizoctonia* on a new medium with pectin as the sole carbon-source. These observations directed the writer's attention to pectic enzymes in the synergistic relation of these two fungi in the rotting of potato tubers (Elarosi, 1957*a*), and their growth in culture media (Elarosi, 1957*b*).

The several pectic enzymes, their terminology, and their mode of action,

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have been dealt with by different authors (Brown, 1915; Davison and Willaman, 1927; Kertesz and McColloch, 1950; Reid, 1950*a*; Lineweaver and Jansen, 1951; Roboz, Barratt, and Tatum, 1952; Wood, 1955). The relation between pectic enzyme secretions and parasitic activity is still a matter of controversy. Some authors have found that where definite rotting of the parasitized tissue occurs, pectinase is constantly present (Vasudeva, 1930; Chona, 1932; Mishra, 1953). Other authors, however, have been unable to find any correlation between parasitic activity and enzyme secretion by parasites (Harter and Weimer, 1921; Cole, 1956; Singh and Wood, 1956).

MATERIALS AND METHODS

Crude enzyme extracts were prepared as follows. Potato plugs, 25 mm. in length and 17·5 mm. in diameter, were taken from the medulla of King Edward tubers, and put in 6×1 in. test-tubes in the base of which 0·5 ml. distilled water was placed. After autoclaving at 15 lb. for 15 minutes, the plugs were inoculated with the appropriate fungus or fungi, and incubated for 10 days. Infected plugs were hardened with solid CO_2, ground up in a mortar, and each extracted with 20 ml. distilled water. Mixtures, surrounded with ice, were stirred with an electric stirrer for 1 hour, after which they were, while still cold, centrifuged at 3,000 r.p.m. for 5 minutes. The clear solutions were kept in the deep freezer at -15° C. till required. Control was obtained from autoclaved healthy potato plugs. When purified enzymes were needed, the clear solution was precipitated with 5 times its volume of 95 per cent. ethyl alcohol. The precipitate was separated by centrifuging at 3,000 r.p.m. for 5 minutes. The clear solution was decanted, and the precipitate dissolved in distilled water making the volume of the purified enzyme equal to that of the original crude enzyme. For separating enzymes by the use of partition paper chromatography, the crude enzyme extract was concentrated to one-tenth its original volume by freeze-drying.

Protopectinase activity was estimated by the maceration of potato-tuber discs (Brown, 1915). Discs 17·5 mm. diameter and 500μ thickness were used. 2 ml. of the enzyme solution were added to 5 ml. of McIlvaine's standard buffer solutions (Hodgman, 1948), and 10 discs were placed in each solution. These were examined periodically for maceration. Discs were considered macerated when they did not show any resistance to tearing by slight tension. Enzyme activity was obtained by dividing 1,000 by the time in minutes needed for maceration (Wood, 1955).

The enzyme causing loss of viscosity was estimated by determining the reduction in the viscosity of pectin, applying the method used by Wood (1955). The relative viscosity was obtained by dividing the time required for passage of the pectic enzyme by the time required for passage of water at the same temperature. Control values were obtained by using heat deactivated enzymes. Pectinesterase was estimated by Kertesz's (1937) method at intervals up to 5 hours.

For paper partition chromatography, Whatman No. 1 filter paper was used in 10×46 cm. strips. The concentrated enzymes were loaded along a line 10 cm. from one end of the filter paper. The developing solution used was 50 per cent. aqueous acetone. Each chromatogram was run overnight in the cold room (0°–5° C.), and dried in front of a fan in the cold room. Then it was cut horizontally into 10 equal bands, beginning after the loading-point, and ending with the front. The loading line was cut separately, and a control band was cut from the part before the loading-point. Each band, cut into small pieces, was eluted in 5 ml. buffer solution of pH 6·5 for 24 hours in the cold room. The protopectinase activity of the eluted bands was estimated by Brown's maceration method (Brown, 1915).

Chromatograms of pectin degradation were allowed to run overnight at room temperature using sec. butyl alcohol, glacial acetic acid, and water in the proportions 5:1:2 respectively, as solvent solution, after which they were air-dried, then sprayed with benzidine solution prepared according to Horrocks (1949), and dried at 105° C. for 15 minutes.

The pectin used throughout the experimental work was 240 apple pectin. In the experiments on degradation of pectin, purified pectin was prepared from the apple pectin using Jermyn and Tomkins's (1950) method.

EXPERIMENTAL RESULTS

(*a*) *Effect of pH on the activity of enzymes causing maceration and loss of viscosity*

Crude enzymes from potato plugs infected with *Rhizoctonia solani* and *Fusarium solani*, individually and together were tested. The pH range of test solutions was obtained by adding 2 ml. of enzyme to 5 ml. of buffer solution of the required pH, using as a control an extract of uninfected potato plug.

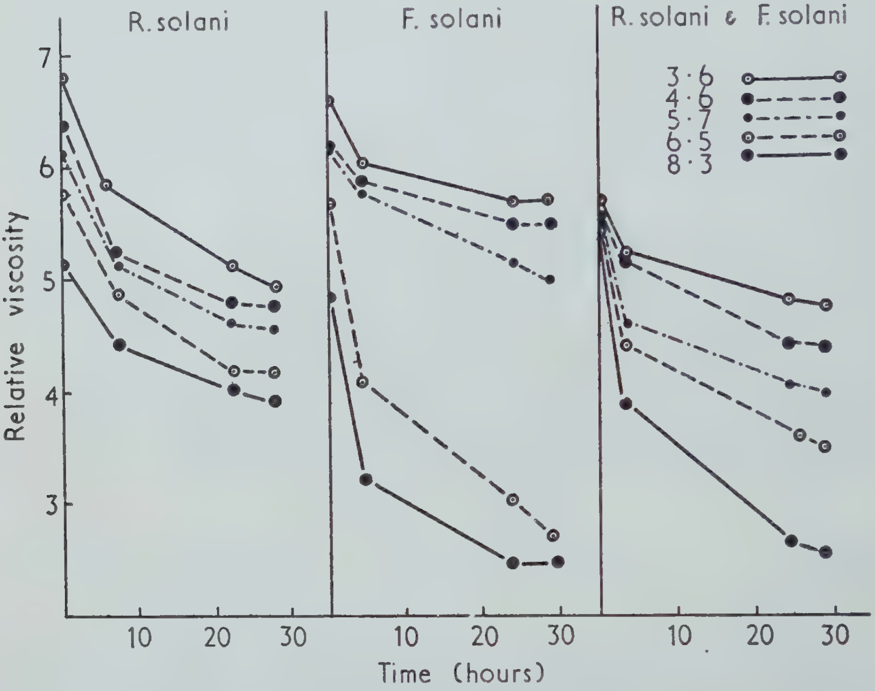
TABLE 1

The Effect of pH on Protopectinase Activity

	Activity			
pH	*R. solani*	*F. solani*	Double infection	Control
3·6	*2·41	9·61	8·93	< 0·56
4·6	2·38	12·05	12·19	< 0·56
5·7	2·74	14·49	17·24	< 0·56
6·5	3·59	28·57	37·04	< 0·56
8·3	3·45	19·61	23·81	2·63

* Figures represent $\frac{1,000}{\text{maceration time (min.)}}$

The experimental results (Table 1) show that with each of the three extracts, the greatest activity was obtained at pH 6·5. In the controls, no maceration was obtained in a 24-hour period except at pH 8·3, where it occurred in 6·20 hours. The *Rhizoctonia* extract showed poor activity. However, when *Rhizoctonia* and *Fusarium* had grown together on the plug, the activity of the enzyme obtained was more pronounced than after infection with *Fusarium* alone, indicating a synergistic phenomenon. This effect was most evident at the optimum pH value, and not at all at values 3·6 and 4·6.



TEXT-FIG. 1. Effect of pH value on the loss of viscosity of 1% aqueous pectin solution, by fungal crude enzymes.

TABLE 2

Effect of pH on the Activity of Crude Enzyme Causing Loss of Viscosity of 1% Pectin Solution

	Time (hr.) for 25% loss of viscosity		
pH	*R. solani*	*F. solani*	Double infection
3·6	21·30	> 30·00	6·00
4·6	14·30	> 30·00	5·30
5·7	12·00	27·30	2·30
6·5	8·00	2·30	2·30
8·3	18·30	2·30	3·00

The changes in viscosity due to the addition of the crude enzyme to 1 per cent. aqueous pectin solution at different pH values are illustrated in Text-fig. 1. The times required to get 25 per cent. loss of viscosity were estimated from these graphs and are given in Table 2. The greatest activity of *Rhizoctonia* enzyme was obtained at pH 6·5. The optimum for *Fusarium* extract lay between 6·5 and 8·3, and for the double infection extract between 5·7 and 6·5. Double infection brought about more rapid loss of viscosity than did either of the two fungi alone. Thus, the protopectinase and 'loss of viscosity' enzyme were similar as far as optimum pH was concerned. However, the synergistic effect between the two fungi on the 'maceration' enzyme activity reached its greatest expression in the range 5·7 to 8·3, while for the 'loss of viscosity' enzyme the synergism was greatest at pH values 3·6 to 5·7.

(*b*) *Effect of different combinations of fungi on the activity of protopectinase enzyme*

As the 'maceration' enzyme produced by *Rhizoctonia solani* and *Fusarium solani* together was more active than that produced by either alone, the activities of the crude enzymes obtained from different fungi, individually and in combinations with *Rhizoctonia solani*, were studied. The enzymes were prepared as before. The fungi studied here were *Fusarium solani*, *F. roseum* (Dis.), *F. roseum* (Ros.), *Cylindrocarpon radicicola*, *Phoma foveata*, and *Rhizoctonia solani* (Elarosi, 1957*b*). The activities of the enzymes were estimated using pH 6·5 buffer solution, which had been found useful for *R. solani* and *F. solani* enzymes.

The previous result of increased activity of protopectinase enzyme obtained from double infection with *Rhizoctonia* and a test fungus was obtained here with the three Fusaria used. *Cylindrocarpon* enzyme was more active when obtained from a pure culture than from the double infection. *Phoma* enzyme did not show any clear effect, whether obtained from pure culture or a mixed one. These results help to explain those of the effect of individual and mixed cultures on fungal attack of potato discs (Elarosi, 1957*b*). In the present experiment, mixed inoculation with *Rhizoctonia* and *Fusarium* spp. yielded more active enzymes than with either fungus alone. In the corresponding cases in the earlier paper, more vigorous attack on potato discs resulted from a double infection. Enzyme activities are thus correlated with the extent of attack on potato tissue by these fungal combinations. *Cylindrocarpon* and *Rhizoctonia* previously showed limited attack on potato discs, either alone or together, no mutual stimulation occurred, nor was there any intermingling of the hyphae of the two fungi. The activity of the enzyme from the double infection treatment was less than half the total of the activities of the enzymes from both fungi alone. *Phoma* attacked potato discs much more vigorously than did *Rhizoctonia*. It is, therefore, possible that in the present experiment, in the double infection, *Phoma* overgrew *Rhizoctonia*, attacking the potato tissue, and producing its enzyme, irrespective of the presence of *Rhizoctonia*. This would explain the fact that no difference was observed between the activities of the enzymes from the pure inoculation with *Phoma*, and the double-infection treatment.

A further experiment included a repetition of the foregoing, omitting *Phoma foveata*; in addition, the activities of enzymes from individual fungi mixed with *Rhizoctonia solani* enzyme were studied. These were prepared by mixing equal amounts of the two crude enzymes, and examining after adding 2 ml. of the mixture to 5 ml. buffer solution. The activities of enzymes and their mixtures, presented in Table 3, show that mixed enzymes from pure cultures of *Fusarium solani* and *Rhizoctonia solani* gave even better protopectinase activity than the enzyme from the double infection. Mixtures of enzymes from the other two fusaria did not show clear activity over those from the individual fungi. *Cylindrocarpon* showed the same trend in the mixture as in the double infection, i.e. weaker activity than either enzyme alone. These results are explicable on one of the two assumptions.

TABLE 3

Effect of Enzymes of Rhizoctonia solani *in a Double Infection and in Mixtures with Other Enzymes on Protopectinase Activity*

	Activity of		
	Fungus alone	Double infection	Mixed enzymes
R. solani	4·33	—	—
F. solani	24·39	26·32	31·25
F. roseum (Dis.)	6·85	8·33	6·13
F. roseum (Ros.)	4·50	8·40	5·43
C. radicicola	6·71	3·98	3·00

1. *Rhizoctonia* enzyme activates *Fusarium* enzymes and retards the *Cylindrocarpon* enzyme.

2. Enzyme activities are affected by the dilution and also depend on the source of enzyme. For instance, if it is assumed that dilution of the enzymes of *Fusarium solani* and *Rhizoctonia solani* to half the original concentration (i.e. when each was used alone) did not change their activity, then the resultant mixed enzyme would have the additive activity of both of them. According to Table 3, the activity of mixed enzyme in this instance ought to be,

$$4{\cdot}33 + 24{\cdot}39 = 28{\cdot}72.$$

But, if we assume that the activity of each is reduced to one-half by dilution to one-half, so the activity of the mixture would be,

$$\frac{4{\cdot}33}{2} + \frac{24{\cdot}39}{2} = 14{\cdot}36.$$

In order to know which of the two assumptions given above obtains, it was necessary to investigate the effect of dilution of the enzymes on their activities.

Three concentrations of the crude enzymes were examined for their maceration activities. The following were added to 5 ml. 6·5 buffer solution.

a. 2 ml. crude enzyme.
b. 1 ml. crude enzyme plus 1 ml. distilled water.
c. ½ ml. crude enzyme plus 1½ ml. distilled water.

The data obtained (Table 4) show that the activity of diluted solutions was less than that of concentrated ones, in all the fungi examined, and the relative reduction in activity differed in each case.

If we consider the enzyme of *Rhizoctonia* individually (Table 3), and apply the effect of dilution of its enzyme preparation from 2/7 to 1/7 as shown in Table 4, the activity after dilution ought to be,

$$4{\cdot}33 \times \frac{< 0{\cdot}69}{3{\cdot}13} = < 0{\cdot}95.$$

In the same manner the activities of the enzymes of other fungi ought to be,

13·91 for *Fusarium solani*,
3·32 for *Fusarium roseum* (Dis.),
2·68 for *Fusarium roseum* (Ros.),
3·26 for *Cylindrocarpon radicicola*.

In the mixed enzymes from *Rhizoctonia* and each of the others, where each preparation is present at the 1/7 level, and assuming that no activation or retardation resulted from mixing, and knowing that the activity of *Rhizoctonia* preparation is in the range 0·00 to 0·95, the activity of the mixed cultures ought to be:

13·91 to 14·86 with *Fusarium solani*,
3·32 to 4·27 with *Fusarium roseum* (Dis.),
2·68 to 3·63 with *Fusarium roseum* (Ros.),
3·26 to 4·21 with *Cylindrocarpon radicicola*.

When these figures are compared with those of Table 3, it is clear that the calculated figures for the three fusaria are smaller than the observed ones. There must, therefore, be an activating effect as a result of mixing the enzymes. Because the calculated figure for the mixed *Cylindrocarpon* enzyme is slightly higher than the observed one, it may be that mixing the two enzyme preparations causes a slight deactivation, or else, since the difference between the two figures is small, the mixing of enzymes may have no effect on the activity of both components, the difference in this case being due to experimental error.

TABLE 4

The Effect of Dilution on the Maceration Activity of the Crude Enzyme

	Activities at concentration		
	2/7	1/7	½/7
R. solani	3·13	<0·69	<0·69
F. solani	17·54	10·00	6·80
F. roseum (Dis.)	3·61	1·75	0·74
F. roseum (Ros.)	5·55	3·30	1·05
C. radicicola	4·26	2·07	0·79

As it is known that the properties of protopectinase from one source may differ from that of another, it is possible that the kind of 'maceration' enzyme differs in each preparation, the mode of reaction and the sites of attack being specific for each kind. On this assumption, protopectinase may be considered as a group of enzymes, some members of which may be lacking in one preparation, and others in another. On this basis, it is suggested that when a stimulation between two macerating enzymes exists, the kind of macerating enzymes present in the two preparations may be different, and the preparations will complement each other in the mixture. This could explain the non-additive effect of the increase in activity on mixing the preparation from *Rhizoctonia* and the different *Fusarium* spp. On the same grounds, when stimulation does not occur, and no clear retardation is found, the kind of macerating enzymes may be regarded as nearly the same. *Rhizoctonia* and *Cylindrocarpon* together afford an example of this.

TABLE 5

The Effect of Cylindrocarpon radicicola *in Mixtures with Fusaria Enzymes on Protopectinase Activity*

	Observed activity		
	Fungus alone	Mixed enzyme	Expected activity
C. radicicola . .	3·31	—	—
F. solani . .	15·63	17·54	10·52
F. roseum (Dis.) .	3·83	4·46	3·47
F. roseum (Ros.) .	3·81	4·46	3·88

In a trial to test the conclusion that *Rhizoctonia* and *Cylindrocarpon* enzymes are nearly identical, activities of mixtures of each of the *Fusarium* enzymes with the *Cylindrocarpon* enzyme were tested. The expected maceration activities were calculated, on the assumption that the enzyme activities are reduced by dilution in the ratios obtained before (Table 4). Here the mixtures showed higher activities than the expected ones (Table 5), indicating that the effect of mixing *Cylindrocarpon* enzyme with enzymes of *Fusarium* spp. was more than additive, i.e. exhibiting the stimulation effect previously obtained with *Rhizoctonia*. The evidence from this experiment serves to verify the hypothesis that the kind of macerating factor formed by *Rhizoctonia* is similar to that formed by *Cylindrocarpon*, and that these two are different from those produced by *Fusarium* spp.

TABLE 6

Effect of Presoaking the Potato Discs in Crude Enzymes of R. solani *and* C. radicicola *respectively, on their Maceration by* F. solani *Enzyme*

		Time of maceration (min.)		Activity	
Pretreatment	Maceration treatment	Observed	Expected	Observed	Expected
—	*Rhizoctonia* enzyme	> 540	—	< 1·85	—
—	*Cylindrocarpon* enzyme	> 540	—	< 1·85	—
—	*Fusarium* enzyme	72	—	13·88	—
10 min. *Rhizoctonia* enzyme	*Fusarium* enzyme	62	> 70·67	16·13	< 14·15
30 min. *Rhizoctonia* enzyme	*Fusarium* enzyme	51	> 68·00	19·61	< 14·71
60 min. *Rhizoctonia* enzyme	*Fusarium* enzyme	50	> 64·00	20·00	< 15·62
10 min. *Cylindrocarpon* enzyme	*Fusarium* enzyme	58	> 70·67	17·24	< 14·15
30 min. *Cylindrocarpon* enzyme	*Fusarium* enzyme	55	> 68·00	18·18	< 14·71
60 min. *Cylindrocarpon* enzyme	*Fusarium* enzyme	51	> 64·00	19·61	< 15·62

This hypothesis was tested again by studying the effect of pre-soaking potato discs in crude enzymes of *Rhizoctonia solani* and *Cylindrocarpon* *radicicola* for different periods of time, on their subsequent maceration by *Fusarium solani* (Table 6). Series of potato discs were put in enzyme solutions of *Rhizoctonia* and *Cylindrocarpon* at pH 6·5, for 10, 30, and 60 minutes respectively, after which the discs were immersed in *Fusarium* enzyme solution at the same pH, and the times required for maceration in the *Fusarium* enzyme were determined. The maceration times in *Rhizoctonia* and *Cylindrocarpon* enzymes alone were also determined. The expected maceration times were calculated on the assumption that *Rhizoctonia* and *Cylindrocarpon* enzymes are of the same kind as the *Fusarium* enzyme, the calculations being made in the following manner: 10 minutes soaking in *Rhizoctonia* enzyme is equivalent to $10 \times \frac{72}{>540} = < 1{\cdot}33$ minutes soaking in *Fusarium* enzyme. Thus subsequently in *Fusarium* enzyme these discs ought to macerate in

$$72 - < 1{\cdot}33 = > 70{\cdot}67 \text{ minutes.}$$

Expected activities were calculated from the expected maceration times.

Table VI shows that pre-soaking potato discs in *Rhizoctonia* or *Cylindrocarpon* enzymes resulted in a reduction in the time needed for *Fusarium solani* enzyme to macerate these discs, i.e. increased the protopectinase activity of the *Fusarium* enzyme. In fact, the assumption that protopectinases produced by the different fungi are similar is not supported by the results of this and the previous experiments, and it is concluded that at least two different macerating enzymes occur, and that each fungus is specific in respect of the kind it produces.

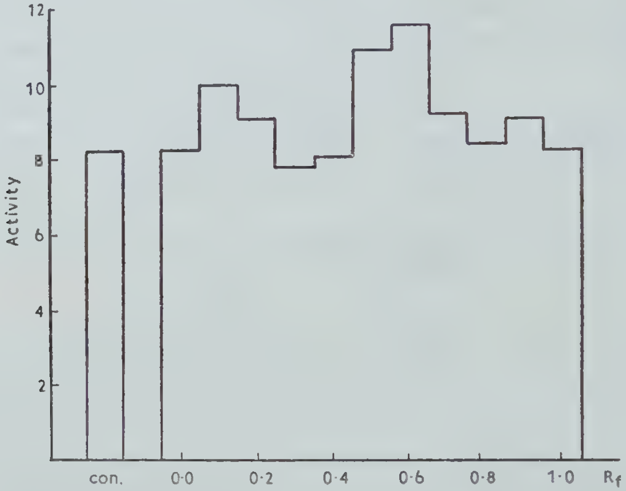
(*c*) *Separation of the components of* Rhizoctonia solani *and* Fusarium solani *enzymes, using paper partition chromatography*

Crude enzymes were concentrated to 1/10th the original volume by the freeze drier, and 0·5 ml. of the concentrated *Rhizoctonia* enzyme and 0·3 ml. of the concentrated *Fusarium* enzyme were loaded on separate chromatograms. After running and drying the chromatograms, bands were cut and eluted in 5 ml. of 6·5 pH buffer solution. To each of the eluates 2·0 ml. of the crude enzyme of the other fungus were added, then potato discs were soaked in the solution, and maceration times observed.

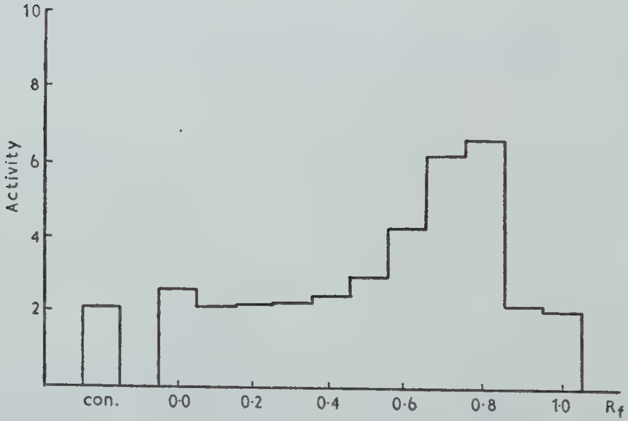
Text-fig. 2 shows the protopectinase activities of the *Fusarium* enzyme plus different eluates of the *Rhizoctonia* enzyme chromatogram. It is apparent that bands of R_f values equal to 0·1, 0·2, 0·5, and 0·6 of *Rhizoctonia* enzyme had a marked stimulating effect on the *Fusarium* enzyme. Text-fig. 3 shows the activities of the *Rhizoctonia* enzyme to which different bands from the *Fusarium* chromatogram had been added. It is seen that eluates of R_f values equal to 0·7, 0·8, and in a lesser degree 0·6, of *Fusarium* enzyme possess a stimulatory effect on *Rhizoctonia* enzyme.

It seemed valuable to study the effect of combining different stimulatory bands from chromatograms of *R. solani* and *F. solani* enzymes. Bands 0·1 and 0·2 of *Rhizoctonia* enzyme were eluted together, and also bands 0·5 and

0·6; similarly, bands 0·7 and 0·8 of *Fusarium* enzyme were eluted. The eluates from each group of two bands were made in 12 ml. of 6·5 pH buffer solution. After the 24-hour elution period the pieces of chromatograms were taken out of the buffer solutions, but the contained liquid squeezed back into the eluted solution, and the solutions only used for determining maceration activities. In each treatment, the total amount of eluted solution used for maceration was 4·8 ml., this being the sum of equal amounts of the components (Table 7). The control (treatment 8) was obtained by examining the maceration activity of an eluted band cut from the region before the loading point of the extract. The activities of the macerating enzymes were taken after a



TEXT-FIG. 2. Protopectinase activity of *Fusarium* enzyme, with added eluates of differing R_f from *Rhizoctonia* enzyme chromatogram.



TEXT-FIG. 3. Protopectinase activity of *Rhizoctonia* enzyme with added eluates of differing R_f from *Fusarium* enzyme chromatogram.

period of 24 hours; in this period of time zero activity was obtained from discs in the control treatment.

On account of the poor activities obtained, it was difficult to find the effect of different dilutions on activity, so the theoretical activities were calculated on the basis that dilutions affect activity in an arithmetical ratio. Comparing the individual groups of bands alone, it is seen that bands 0·7 and 0·8 of *Fusarium* showed maximum maceration effect, while low activity occurred in bands 0·5 and 0·6 of *Rhizoctonia*, and none at all in bands 0·1 and 0·2 of *Rhizoctonia*. Adding either of the two groups of bands of *Rhizoctonia* to those of *Fusarium* stimulated activity of the *Fusarium* eluted solution. Bands 0·1 and 0·2 were more effective in this respect than bands 0·5 and 0·6, but the combination of all three groups of eluted solutions was more effective than any two (Table 7). Thus, it is clear that bands 0·1 and 0·2 of *Rhizoctonia*, although by themselves not showing any maceration activity in 24 hours, do, when present with other bands, stimulate the macerating activity of these (treatments 2, 5, and 6).

TABLE 7

Effect of Mixed Elutions of the Active Chromatogram Bands on Protopectinase Activity

	Bands from *Rhizoctonia*	Bands from *Fusarium*	Amount of sol. from each band (ml.)	Activity after 24 hrs. Observed	Expected
1.	0·1, 0·2	—	4·8	0·00	—
2.	0·1, 0·2	0·7, 0·8	2·4	4·48	2·14
3.	0·5, 0·6	—	4·8	0·61	—
4.	0·5, 0·6	0·7, 0·8	2·4	3·91	2·44
5.	0·1, 0·2, and 0·5, 0·6	—	2·4	0·61	0·31
6.	0·1, 0·2, and 0·5, 0·6	0·7, 0·8	1·6	4·00	1·62
7.	—	0·7, 0·8	4·8	4·27	—
8.	—	—	4·8	0·00	—

(*d*) *Detection of pectinesterase and polygalacturonase*

The fact that only the de-esterified portions of pectic substances are susceptible to the action of polygalacturonase (Jansen and MacDonnell, 1945) suggested that one of the macerating bands of *Rhizoctonia* might be pectinesterase, and that the other might be polygalacturonase.

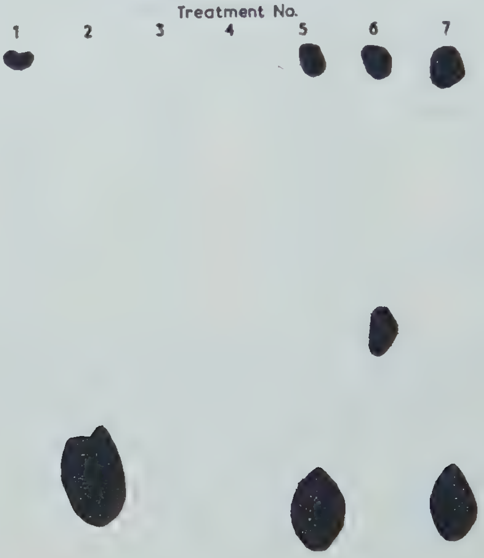
Pectinesterase activity of the crude enzymes was estimated by titrating pectin solution treated with the enzyme against 0·1 N. sodium hydroxide. The following amounts of methoxyl (CH_3O) were split per 1·0 ml. in 5 hours:

3·72 mg. by *Rhizoctonia* enzyme,
0·31 mg. by *Fusarium* enzyme,
0·15 mg. by uninoculated potato tuber extract.

Using Reid's (1950*b*) method, the enzyme chromatograms also were tested for pectinesterase and polygalacturonase activities, but no clear positive results were obtained.

(*e*) *Degradation of pectin solution by purified enzymes*

Up to this stage, different protopectinases have been demonstrated, and different components have appeared. It was suggested that pectin degradation by purified enzymes may throw some light on the protopectinase components and their mode of action. Purified enzymes of *Rhizoctonia solani* and *Fusarium solani* were added, individually and in combination, to a 1 per cent. pure pectin solution at the ratio (v/v) of 1 to 5. In a control treatment a solution of protein precipitate from healthy potato tissue was substituted for the enzyme. Solutions were incubated at 25° C. for 24 hours. Thereafter spots of the solutions were placed 1·9 cm. apart on the base line of a strip of filter paper as follows:



Text-fig. 4. A tracing of a chromatogram of pectin degradation by fungal enzymes (half size). For explanation see text.

(1) control;
(2) galacturonic acid;
(3) *Rhizoctonia* enzyme;
(4) *Fusarium* enzyme;
(5) pectin solution plus *Rhizoctonia* enzyme;
(6) pectin solution plus *Fusarium* enzyme;
(7) pectin solution plus *Rhizoctonia* and *Fusarium* enzymes.

Chromatograms were run for 20 hours, dried, then sprayed with benzidine, and dried again at 105° C. for 15 minutes. Text-fig. 4 shows the developed chromatogram pattern. Brown spots appeared at the base line of treatments 1, 5, 6, and 7, nothing equivalent being seen in treatments 2, 3, and 4. Other brown spots developed in front of the base line, at R_f 0·22 to 0·28 with galacturonic acid; at R_f 0·24 to 0·29 in treatment no. 5; at R_f 0·15 to 0·18 in treatment no. 6; and at R_f 0·24 to 0·28 in treatment no. 7. No spots were found in front of the base line in treatments 1, 3, and 4. The reducing sugar which appeared as the result of adding *Rhizoctonia* enzyme, or a mixture of *Rhizoctonia* and *Fusarium* enzymes, to pectin solution is equivalent to galacturonic acid. It is suggested that the compounds causing the spot opposite the *Fusarium* enzyme treatment (no. 6) is an intermediate stage in the degradation of pectin. As this suggested intermediate stage was not present in the mixed treatment (no. 7), it is further surmised that the *Rhizoctonia* pure enzyme contains two enzymes, one converting the pectin directly to galacturonic acid, the other converting the intermediate stage formed by *Fusarium* to galacturonic acid. An alternative suggestion is that the first enzyme breaks down the pectin to the intermediate stage formed by *Fusarium*, while the second acts simultaneously on this intermediate, degrading it to galacturonic acid, so that no detectable level of the intermediate occurs in the presence of this enzyme.

DISCUSSION AND CONCLUSION

(*a*) *Pectic enzymes*

The role of pectic enzymes in fungal pathogenesis cannot be overlooked. For a fungus to establish and maintain an intercellular habit of growth, the ability to digest the middle lamella would appear to be important; for it to grow intracellularly, the hyphae must pass through the host cell walls, either by enzymic digestion or mechanically (in some cases the pits may serve as entrances for hyphae). As protopectin is a major constituent of the host cell walls, it is assumed that a successful parasite may have, among other factors, the ability to secrete protopectinase.

The structure and composition of protopectin are matters of dispute (Kertesz and McColloch, 1950). The chemical constitution of protopectinase, and its specific mode of action, have not been reported. The possibility that there may be more than one kind of protopectinase has been suggested. Thus, the characteristics of the enzyme have been found to differ with the fungal source, with the substratum used, and also with the extra- or intracellular nature of the enzyme (Chona, 1932; Menon, 1934; Tribe, 1955). Chona (1932) thought his results indicated the probable existence of a limited number of distinct types of protopectinase. He did, however, suggest an alternative explanation, namely, that the enzyme is the same in all cases, but that the details of its behaviour are modified by the presence of other substances. Fernando and Stevenson (1952) thought that protopectinase activity might be altered through interference by other enzymes. Some authors have doubted the existence of protopectinase enzyme as an entity (Kertesz and McColloch, 1950; Linweaver and Jansen, 1951; Winstead and Walker, 1954). An extension of this idea (Winstead and Walker, 1954) is that the dissolution of the middle lamellae by fungal attack is due to the double effect of pectinesterase and polygalacturonase when produced in large quantities. Pectinesterase is thought to diffuse ahead of the mycelium inside the host tissue, and to split the methoxyl groups from the pectin molecule, the resulting simpler molecule providing the substrate for polygalacturonase, which converts the pectate or pectic acid into galacturonic acid. More recently, a resemblance in properties between protopectinase and polygalacturonase has been demonstrated (Singh and Wood, 1956), and also between protopectinase and depolymerase (Wood, 1955).

From the foregoing literature it appears to the author that polymerase or polygalacturonase plays a part in maceration of cells. As the chemical constitution of protopectin is a matter of supposition, it cannot at present be categorically stated that protopectinase is identical with polygalacturonase or demethylase. In the light of the work discussed here, a theory is put forward for further examination:

> *Protopectinase is not one enzyme but a system of related enzymes which may be successively evoked, catalysing the breakdown of protopectin and ending by the depolymerization of the parent insoluble pectin to small soluble units. The specific property and activity of the protopectinase will be dependent on the kind and amounts of its enzyme components present, and their relative proportions.*

According to this theory, polygalacturonase or depolymerase, which performs the depolymerization stage, may be considered as a probable part of the protopectinase complex. De-methylated pectins are more easily acted upon by polygalacturonase than are the methylated ones; consequently the presence of pectinesterase in the enzyme complex would facilitate the maceration process in the presence of polygalacturonase.

The finding of Ayres, Dingle, Phipps, Reid, and Solomon (1952) showed that the problem of the relations between pectic enzymes was more complex than has been thought. They showed that in *Aspergillus foetidus* cultures there are three enzymes capable of degrading pectin and pectic acids. One of them can attack pectin, as well as pectic acids, but without the formation of galacturonic acid. The second attacks only pectic acid, but can continue the reaction with the final formation of galacturonic acid. The third, acting on the polyuronides, degrades them to galacturonic acid. It seems to the writer that the first enzyme is equivalent to pectin depolymerase, and the second to polygalacturonase both in mode of reaction and pH optima (Roboz, Barratt, and Tatum, 1952). However, the first enzyme differs in pH optimum from the 'bacterial depolymerase', studied by Wood (1955).

In the present work, the mixing of *Rhizoctonia* and *Fusarium* crude enzymes resulted in increased activity as measured by maceration. *Rhizoctonia* enzyme was separated by the use of paper partition chromatography into two parts, each of which affected the *Fusarium* enzyme differently. Pectinesterase activity was demonstrated in *Rhizoctonia* crude enzyme, but was not clearly marked in the *Fusarium* crude enzyme.

The study of the effect on pectin of purified enzymes obtained from the two fungi showed that pectin can be degraded to galacturonic acid by the enzymes of *Rhizoctonia*, and by the mixed enzymes, but not by the *Fusarium* enzyme. This was attributed to activity of two relevant enzymes in *Rhizoctonia*, *Fusarium* having only one. The observed facts of the *Rhizoctonia* enzyme complex can be explained by either of two alternative possibilities.

1. One enzyme degrades pectin directly to galacturonic acid, and the other degrades the polyuronides, formed by *Fusarium* activity, to galacturonic acid. On this hypothesis the same enzyme may act on the two substrata.

$$\text{Pectin} \xrightarrow{\text{Enzyme } \alpha} \text{Galacturonic acid}$$

$$\text{Polyuronides} \xrightarrow[\text{or Enzyme } \beta]{\text{Enzyme } \alpha} \text{Galacturonic acid}$$

2. One enzyme degrades pectin to polyuronides and the other degrades the polyuronides to galacturonic acid.

$$\text{Pectin} \xrightarrow{\text{Enzyme } \alpha} \text{polyuronides} \xrightarrow{\text{Enzyme } \beta} \text{Galacturonic acid}$$

Since none of the enzymes agrees in mode of reaction with polygalacturonase or pectin depolymerase, the first suggestion may be discarded. There is, furthermore, evidence which supports the second suggestion. There is a close resemblance between its first enzyme and both pectin depolymerase and the first enzyme of Ayres *et al.* (1952) in the mode of reaction. The second enzyme agrees in mode of reaction with the third of Ayres *et al.* According to this second suggestion, the *Fusarium* extract contains an enzyme equivalent to pectin depolymerase. However, despite the fact that *Rhizoctonia* extract contains enzymes capable of forming the whole process of degradation as far as galacturonic acid, its maceration activity is weak, probably because of a low enzyme concentration.

(*b*) *Synergism in parasitism*

In this section of the discussion, the associations between *Rhizoctonia solani* and *Fusarium solani*, in rotting potato tubers (Elarosi, 1957*a*), will be discussed in the light of the present work and a previous one (Elarosi, 1957*b*).

When *Fusarium solani* attacks living potato first, it grows slowly and is restricted by cork formation (Elarosi, 1957*a*). Increase in pH and formation of a staling factor inhibit further *Fusarium* growth, and also any following *Rhizoctonia solani* growth (Elarosi, 1957*b*). When *Rhizoctonia* alone invades potato it colonizes but few host cells, filling them with sclerotial masses (Elarosi, 1957*a*). The reason for this restricted growth may be due to successful host-resistance. *Fusarium* following *Rhizoctonia* infection advances past *Rhizoctonia*, after which *Rhizoctonia* resumes its parasitic activity. Working from the results described in the present paper the following hypothesis is put forward for further explanation.

When Fusarium *is inoculated in a site previously inoculated with* Rhizoctonia, *it grows in the intercellular spaces, hydrolysing the pectin moieties of the middle lamellae into polyuronides.* Fusarium *continues growth till it advances past the* Rhizoctonia. *The latter follows* Fusarium *in the intercellular spaces, and thus completes the breakdown of the middle lamellae-pectin to galacturonic acid, which can be used by the two fungi. This process thus removes the polyuronides, the intermediate stage of pectin degradation, which may have a retarding effect on the pectin enzyme reaction and, consequently, on the growth of* Fusarium. *The presence of* Rhizoctonia, *therefore, may activate the reaction catalysed by the* Fusarium *enzyme, and thus restores the growth potentialities of* Fusarium. *Also, after the dissolution of the middle lamellae, it becomes easier for* Rhizoctonia *to break through the host cell walls. Therefore* Rhizoctonia *may now advance easily without the need to accumulate in each cell before breaking through to the next. There is also the possibility that the pectinesterase secreted by* Rhizoctonia *may diffuse in advance of its growth and de-methylate the pectin of the middle lamellae, thus providing an easier substrate for* Fusarium *to hydrolyse.*

The literature on *Rhizoctonia* and *Fusarium* potato-tuber rots contains instances of probable associations between *Rhizoctonia solani* and various species of *Fusarium*, which can be interpreted in the light of these studies. Carpenter (1915) isolated *Rhizoctonia* sp. and different species of *Fusarium* from 'jelly-end rot' of potato tubers. Pratt (1916) thought that *Fusarium* spp. were responsible for this disease. Shapovalov (1922) reported that it is difficult to induce *R. solani* to decompose any appreciable quantity of normal, healthy potato-tuber tissues, when inoculated from pure cultures; the only way in which he obtained 'jelly-end rot' by *R. solani* was to inoculate the stem-ends of abnormal, elongated tubers, and inoculate these under alternating high and low temperatures for 10 days. Recently, Friedman and Folsom (1953) isolated *Rhizoctonia* spp., and *Fusarium* spp., and several types of bacteria from 'jelly-end rots', yet none of these singly reproduced the disease symptoms. Thatcher (1942), by pathogenicity inoculation tests, showed a stem-end rot of potato tubers which was caused by *Rhizoctonia solani* to be strongly suggestive, in its external symptoms, of the stem-end rot caused by *Fusarium solani* var. *eumartii*.

From these observations it appears to the writer that *Rhizoctonia solani* together with any of several different *Fusarium* spp. may be associated in the development of the 'jelly-end rot' and stem-end rot, in a similar manner to the development of the potato-tuber rot caused by the two fungi in the present work. From the knowledge that the stem-ends of the majority of potato tubers are normally infected with fungi (Chamberlain, 1935), and that the majority of these fungi are *Fusarium* spp., and a few are *Corticium vagum* (*R. solani*), it can be suggested that infection by either fungus, i.e. *Rhizoctonia* or *Fusarium*, may activate the other if it is present in the tuber. Thus, the two fungi can work together in establishing the disease. The activation of a dormant fungus as suggested here resembles the resumption of activity by fungi established in stored fruits as latent infections (Baker and Wardlaw, 1937). If this suggestion is verified, it would explain why the two fungi have been isolated frequently from 'jelly-end rot', why inoculation with *Fusarium* spp. in some cases and with *Rhizoctonia* in others has resulted in the same disease, and also why in other cases none of the isolates individually was able to cause the typical symptoms of the disease. Research along the line of this hypothesis may throw some light on the parasitism in these cases, and perhaps indicate parallelism with the present phenomenon, showing it to be of a wider occurrence.

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The Effect of Removal of the Root-System of Barley on the Production of Ears

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With two Figures in the text

Abstract

Complete removal of roots of barley plants, grown in water culture, at a stage before the plants have attained their maximum shoot number, results in a significantly greater number of ears at harvest. If the roots are excised when shoot number is beginning to decline the number of ears is decreased.

The exact cause of the phenomenon is not known but it is suggested that it may be due to a temporary diversion of carbohydrate to the shoots when the roots are excised.

THE effect of removing different proportions of the root system of plants such as barley and tomato on the subsequent growth of root and shoot has already been described (Humphries, 1958). These experiments were of short duration and included no observations of the effect of root removal on the final development of the plant. The present account is concerned with the influence of removal of the whole root system of barley, grown in water-culture, at successive stages of development on total dry weight and on the number of ears formed at maturity. The experiments were performed in two seasons.

Experimental

A mildew-resistant variety of barley (B. 2118) was germinated and uniform seedlings transferred to pint bottles containing half-strength Hoagland solution. In all there were 14 groups of 6 bottles, each bottle containing two plants. In the first season, seeds were germinated on 24 April and roots removed from the first group of plants 24 days later. After removal of the roots the base of the stem was immersed in fresh culture solution. Another group of plants was harvested at the same time for estimation of the dry weight of root and shoot separately. This process was repeated on separate groups of plants at weekly intervals for a period of 6 weeks. The root system regenerated quickly and no plants were lost by wilting; when wilting did occur it was only temporary. Control plants from which roots were not excised received similar changes of solution. All the plants were allowed to grow to maturity, when they were harvested and separated into roots, straw, and ears.

In the second season roots were removed from plants in 10 bottles and plants in 2 bottles were harvested for dry-matter determination on each occasion. In this experiment counts of shoot number at the successive

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occasions were made. Germination took place on 21 March and the roots were removed from the first group of plants after 40 days' growth, i.e. about 2 weeks later than in the first experiment.

RESULTS

The dry weight of roots and shoots of plants harvested at each successive weekly operation is shown in Fig. 1. The amounts of root removed at particular periods after germination agreed well in the two seasons. There was not such good agreement between the shoot weights. The amounts of root regenerated by the experimental plants, in the first season, between the time of operation and harvest are given in Table 1. The dry weights of regenerated root declined from the time of the first cut onwards but the total amount of root produced, i.e. the original roots together with the regenerated root, was greatest in the last two groups of plants. The total dry weight of straw and

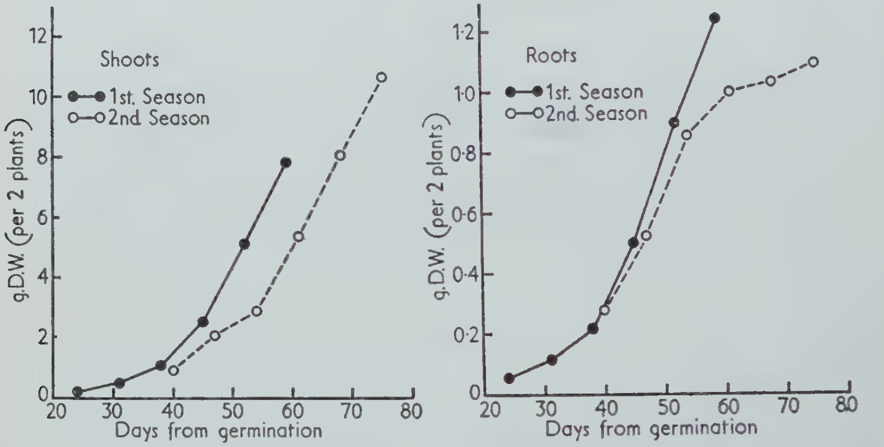

FIG. 1. Increase in dry matter of roots and shoots of barley; ●——● 1st season, ○---○ 2nd season.

TABLE 1

Dry Weights of Roots Removed and of Regenerated Roots at Maturity; Dry Weights of Shoots at Maturity and Total Dry Matter Produced. g. per 2 plants. (1st Season)

Time of root excision (days from germination)	Roots removed	Regenerated roots	Total roots produced	Straw and ears	Total dry matter
24	0·05	1·53	1·58	27·5	29·08
31	0·11	1·40	1·51	25·8	27·31
38	0·26	1·23	1·49	25·8	27·29
45	0·52	0·92	1·44	24·5	25·94
52	0.87	1.05	1.92	26.2	28.12
59	1·29	0·63	1·92	20·2	22·12
Control	—	—	1·23	25·7	26·93
L.S.D.	0·104	0·463	0·486	5·55	6·09

ears produced showed only small variation except in the last group where it was significantly smaller than the first. This applied also to the total dry matter produced.

The average numbers of ears produced by 2 plants in each season are shown in Table 2. In the first season, where the precision was less than in the second, the numbers of ears formed on plants with roots excised at 45 and 52 days after germination were significantly greater than the controls, while in the second season significantly greater numbers of ears were produced by root excision at 40 and 47 days after germination. This suggests that there is a critical period in development during which complete root excision causes the plant to produce more fertile shoots.

TABLE 2

Number of Ears per bottle (2 plants)

Time of root excision (days from germination)

	24	31	38	40	45	47	52	54
1st season	19·7	19·8	22·0	—	22·7	—	23·0	—
2nd season	—	—	—	15·3	—	14·4	—	12·1
				(15·8)		(15·3)		(14·4)

	59	61	68	75	Control	L.S.D.
1st season	13·3	—	—	—	18·3	4·43
2nd season	—	8·7	6·8	6·8	11·5	1·96
		(13·6)	(14·2)	(13·0)	(12·9)	(3·50)

The total shoot number at harvest in the second season is shown in brackets.

Shoot counts made in the second season suggest that root excision at the critical period arrests the decline in shoot number which normally takes place at a certain point in the development of the plant. In Fig. 2 are shown the numbers of shoots per 2 plants recorded at the successive weekly root excisions. The numbers of shoots recorded 8 days after the last root excision and at harvest are also shown. There were 10·5 shoots at the time of the first weekly operation which increased to 26 at the third week and subsequently declined to 17·5 at the sixth week. The control plants had only 12·5 shoots eight days later. This curve (broken line in Fig. 2) gives the changes which took place in normal intact plants during the period. The number of tillers on plants which had their roots excised on the first occasion had increased to 19 after a further 7 weeks, while the number of tillers on intact plants had fallen to 12·5. Root removal on the second occasion caused a slight decline in tiller number (from 19·5 to 18·5) in a 6-week period. When roots were excised on the third and subsequent occasions there was a rapid decline in shoot number which, as may be seen from Fig. 2, followed closely the fall in shoot number of normal plants, so that in these instances the shoot numbers fell approximately to those of the control. Subsequently, there was a slight increase in shoot number at harvest in plants with roots excised on the last 3 occasions, suggesting that root removal had a slight stimulus on tillering. The numbers of shoots at harvest, however, were less than those attained by plants which had the roots excised in weeks 1 and 2, and, more importantly, the proportion of ear-bearing shoots was less in plants with roots excised late than with roots excised early (Table 2). It appears from these observations that a greater number of fertile shoots results only when the excision takes place in the period of increasing shoot number. Root excision during this period apparently lessens the subsequent decline in shoot number.

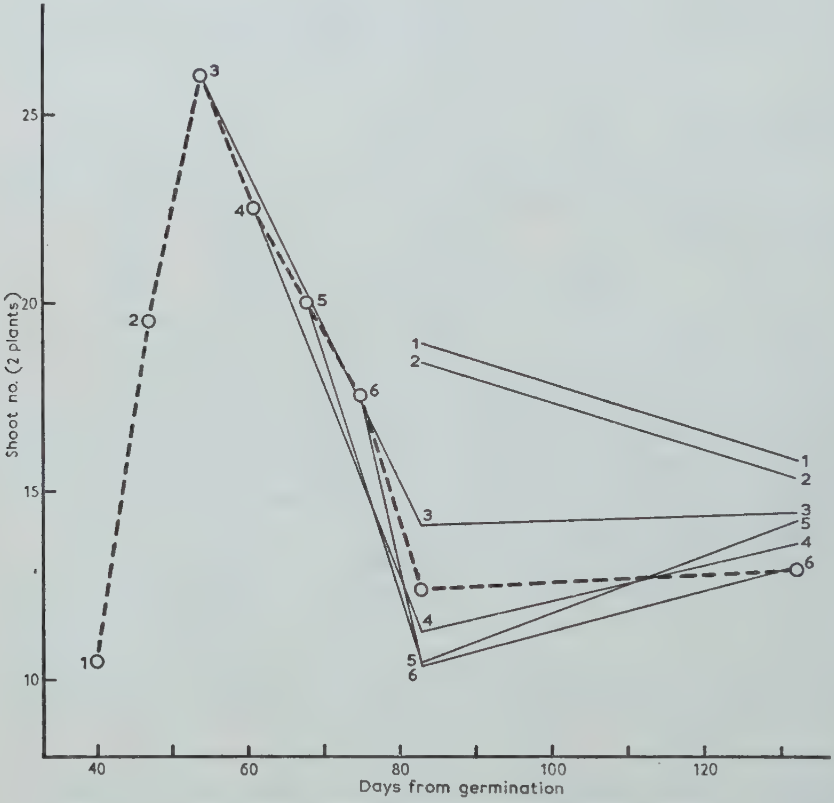

FIG. 2. Changes in shoot number of barley with time; – – – normal plant; 1, 2, &c., roots excised from different batches of plants at weekly intervals.

Possibly the phenomenon described occurs only in particular conditions of mineral nutrition, but insufficient evidence is available to draw definite conclusions. The amount of nitrogen absorbed by the plants during growth was measured in the first season by analysis of the dried material of successive harvests. Comparison with the amounts supplied at each change of solution in the first season showed that none of the plants suffered from nitrogen deficiency (Table 3), although in the interval between 52 days and 59 days from germination the plants absorbed most of the nitrogen supplied. Final contents of nitrogen in the straw and ears of plants with roots cut on the first 5 occasions are very similar in spite of root excision. The amount of nitrogen absorbed by the regenerated root system declines throughout in accord with the decreasing size; root removal therefore made little difference to the final nitrogen content. Results from the first season, therefore, do not suggest that nitrogen nutrition is concerned in the phenomenon. In fact the groups of plants in which the shoot numbers increased after root excision had a slightly decreased nitrogen content. In the second season no nitrogen analyses were made, but it is possible that the plants suffered some nitrogen deficiency, because the first root excision (and therefore the first change of solution) took place later than in the first season. No nitrogen-deficiency symptoms were observed. These observations do not rule out the possibility that nutritional conditions are concerned in the phenomenon, but this must be a matter for future investigation.

TABLE 3

Uptake of Nitrogen (mg.) per 2 plants (1st season)

Time of root excision (days from germination)	At time of root removal			At maturity			Amount absorbed by regenerated root system
	Shoots	Roots	Total	Straw +ears	Roots	Total	
24	10	3	13	315	36	351	341
31	25	5	30	314	35	349	324
38	53	10	63	309	32	341	288
45	101	18	119	295	27	322	221
52	143	25	168	304	25	329	186
59	189	32	221	273	17	290	101
Control	—	—	—	304	32	336	—
L.S.D.	—	—	—	57·7	11·1	—	—

Amount of nitrogen supplied at each weekly interval = 60 mg.

DISCUSSION

No reliable estimate of grain yield was obtained in either season because the hot, dry conditions prevailing in the glasshouse at the time of anthesis resulted in very poor grain formation both in the control and treated plants. The variety used appears to be particularly susceptible to high temperature. There was, however, nothing abnormal in appearance or in size of the ears formed on the treated plants. There is at the moment no evidence to show how root-excision results in a greater number of fertile tillers.

Gregory (1952) has put forward the view that during the development of a plant such as barley, when the demand for mineral elements overtakes supply, 'internal starvation' sets in which is manifested externally by a fall in tillering rate and eventual death of the last-formed tillers. Senescence of these branches sets free nutrients which again become available. This theory does not appear to explain the present observations, since it would be expected that complete removal of roots would hasten the state of 'internal starvation' and cause tillers to die more quickly. The opposite in fact occurs. Another possible explanation is that the competition is concerned with carbohydrate and that complete removal of the root system, although immediately diminishing total shoot growth (Humphries, 1958), may divert carbohydrate normally used in root growth into developing shoots. This would probably give a more adequate supply to ears that normally die when immature through competition with other shoots, and permit them to survive to maturity.

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